COMBINATION THERAPY FOR CANCER

This application claims the benefit under 35 USC 119(e) and under the Paris Convention of Kehoe-Whistance and Maclean, USSN 60/510,516 filed October 14, 2003, atty

5   docket MACLEAN=1 (originally filed in the name of Maclean alone); Kehoe-Whistance, USSN 60/576,624 filed June 4, 2004, atty docket KEHOE=1. Both applications are hereby incorporated by reference in their entirety.

10   **BACKGROUND OF THE INVENTION**

FIELD OF THE INVENTION

The field of the present invention relates to an anti breast cancer therapy. The present invention contemplates methods and compositions for the combination of

15   immunotherapy and anti-hormone (anti-estrogenic steroid) therapy for this purpose.

DESCRIPTION OF THE BACKGROUND ART

Breast cancer

20   Breast cancer is the most frequently diagnosed cancer of women in Canada. Over the course of a lifetime, one in nine women is expected to develop breast cancer and one in twenty seven women will die from it. Despite advances in the diagnosis and treatment of breast

25   cancer, up to 50% of newly diagnosed patients may develop metastases. Metastatic breast cancer has a poor prognosis and is generally considered incurable. Typically, the goals of therapy in the setting of metastatic breast cancer are to control the disease and

30   relieve symptoms as much as is possible to maintain or improve quality of life. It has been estimated that the "residual life expectancy" (the additional time a person with cancer would have lived in the absence of the disease) of a woman with breast cancer is 19.6 years.

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There is a clear and unmet clinical need to develop
effective therapeutics for the prolongation of life and
relief symptoms for women with metastatic breast cancer.

5   *Risk factors in breast cancer*

There a variety of risk factors that are important
in the etiology of breast cancer. These include:
increasing age (risk doubles between the ages of 45 and
65), previous breast cancer in the same patient, family

10   history of breast cancer in a first degree relative
(mother, sister or daughter), a first degree relative
that is pre-menopausal and has bilateral breast cancer, a
first degree relative that is pre-menopausal and has uni-
lateral breast cancer, and a blood relative that is pre-

15   menopausal and has breast cancer. Additional risk
factors include: carriers of mutations of genes such as
BRCA1, BRCA2, p53, PTEN, ATM, a family history of cancer
of the ovary, cervix, uterus or colon, early menarche,
late menopause, nulliparity, first pregnancy over the age

20   of 30, obesity, breast augmentation, oral contraceptives,
hormone replacement therapy (HRT), and radiation
exposure. Diet and alcohol consumption may also affect
the risk of developing breast cancer and this may, in
part, explain the fivefold variation in the incidence of

25   breast cancer that is observed among different countries.

Screening and diagnosis of breast cancer

Screening for the presence of breast cancer can be

30   carried out in a variety of ways, including: beast self-
examination, clinical breast examination, mammography,
and screening mammography. Evaluation of abnormalities
detected during screening can be carried out by: fine-

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needle aspiration, ultrasonography, biopsy, mammography,
stereotactic- and ultrasound-guided core biopsies,
ultrasound- or stereotactic-guided fine-needle
aspiration, magnetic resonance imaging, ultrasound,
5   sestamibi nuclear medicine scanning and positron emission
tomography imaging. The invention is not limited to a
particular method of screening for the presence of breast
cancer.

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Markers of breast cancer
        Her-2/neu and c-erB-2 are receptor protein tyrosine
kinases that members of the epidermal growth factor
receptor (EGFR) family. Over expression of growth factor
15   receptors with homology to EGFR (such as Her-2/neu and/or
c-erB-2) have been found to be associated with a poor
clinical prognosis of beast carcinoma. It has been noted
that those breast carcinomas overexpressing Her2/neu
and/or c-erB-2 tend to lack the estrogen and progesterone
20   receptors, and thus are hormone-therapy less-responsive
(see below), and have a poor clinical outcome. The
invention does not require screening for these markers
of breast cancer.

25   *Staging of breast cancer*
A widely used system to stage breast cancer is the
American Joint Committee on Cancer (AJCC) classification,
which is based on tumor size (T), the status of regional
lymph nodes (N), and the presence of distant metastasis
30   (M), and is referred to as TNM staging. Clinical staging
is performed following physical examination and
radiological studies. Pathologic staging is performed
following surgery for operable breast cancer The stage of

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the cancer may influence the choice of treatment a
skilled clinician may offer to a patient.

Treatment of metastatic breast cancer

5   Due to the heterogeneity of metastatic breast cancer
there are a variety of treatment options for these
patients which include, but are not limited to: surgery,
chemotherapy, radiation therapy, hormonal therapy and
immunotherapy. The preferred treatment regime will

10   depend on factors such as extent of metastases , comorbid
condition and tumour characteristics. Such factors that
contribute to the treatment regime are well known to the
skilled practitioner. Treatment guidelines relating to
various drug products are also well known to the skilled

15   practitioner, and, by way of example, may be found in the
"Compendium of Pharmaceuticals and Specialties (CPS), The
Canadian Drug Reference for Health Professionals", and
other such guides.

20   *Immunotherapy of Breast Cancer*
       WO 03/015796 ("Immunogenic conjugate of carbohydrate
haptens and aggregated protein carrier") describes an
immunotherapy in which an immune response is elicited to
a carbohydrate epitope. In particular, an aggregated

25   STn-KLH (keyhole limpet hemocyanin) conjugate, also known
as the THERATOPE® vaccine, is described.

       The THERATOPE® vaccine developed at Biomira consists
of a synthetic STn hapten conjugate to KLH, delivered in

30   emulsion with an adjuvant. The vaccine used in Phase I
and Phase II clinical trials had a hapten substitution
level that resulted in a sialic acid (NANA) content of
about 2.5 to 3% by weight. While Phase II clinical

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trials were in progress, the conjugation methodology was
improved so that a NANA content of about 7$ could be
achieved. The high conjugation product induced
considerably higher titers of anti-Stn antibody in mice,
5   and significantly higher anti-STn IgG titers in humans,
in a small bridging study. Since higher anti-STn IgG
titers had appeared to be correlated with improved
survival in Phase II clinical trials, a large phase III
clinical trial was initiated using a STn-KLH conjugate
10   with a NANA content of about 7%. Further refinements of
the STn-KLH conjugate are described in PCT/US02/24735,
filed Aug. 5, 2002 (atty docket KRANTZ2.1-PCT).

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SUMMARY OF THE INVENTION

The present invention contemplates the use of anti-hormonal (anti-estrogenic steroid) therapy in combination with immunotherapy as an anti-breast cancer therapy, and kits for practicing said combination therapy. It further relates to methods of identifying patients likely to benefit from such therapy.

The immunotherapy comprises administration of at least one immunotherapeutic agent, which may comprise an immunogen (active immunotherapy), an antibody (passive immunotherapy), or an antigen-activated T cell (passive immunotherapy). The immunogen comprises at least one breast-cancer associated epitope, and the antibody or T cell recognizes at least one breast cancer-associated epitope.

In a preferred embodiment, the breast cancer-associated epitope is a carbohydrate epitope, in particular, the TF, sialyl-TF, Tn or, most preferably, the sialyl-Tn epitope. Even more preferably, the immunogen is an Stn-KLH conjugate. Most preferably, the immunotherapeutic agent is the THERATOPE® vaccine.

The breast cancer-associated epitope may alternatively be a peptide or glycopeptide epitope, in which case it preferably is a MUC1 epitope.

It should be noted that MUC1 comprises Sialyl Tn epitopes as well as peptide epitopes.

By anti-hormonal (anti-estrogenic steroid) therapy is meant therapy which comprises administration of an agent which inhibits an endogenous hormone (a human estrogenic steroid) which, at normal or elevated levels of activity, is a risk factor for breast cancer.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 Survival of THERATOPE® vaccine versus KLH
Control groups for Fully Adjusted Hormone Subset:
5   Original Data. Survival distribution function plotted
against survival time (months).

Figure 2 Median Survival for Fully Adjusted Hormone
Subset as of Update #2. Survival distribution function
10   plotted against survival time (months).

Figure 3 Survival by OSM Response for the Fully Adjusted
Hormone Subset of THERATOPE® vaccine Treated Patients, as
of Update #2. Survival distribution function plotted
15   against survival time (months).

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DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

The present invention relates to the use of anti-hormonal (anti-estrogenic steroid) therapy in combination
5   with immunotherapy (such as with the THERATOPE® vaccine) as an anti-breast cancer therapy.

Estrogenic steroids

The contemplated endogenous human estrogenic
10   steroids are principally the human estrogens 17beta-estradiol, estrone and estriol. We use the term "estrogenic steroid" instead of "estrogen" principally because some scientists use the term "anti-estrogen" to refer to compounds which antagonize estrogen by a
15   particular mechanism. The term "anti-estrogenic steroid" is not limited to any particular mechanism of action.

Hormones and breast cancer

Hormones, such as estrogen, play an important role
20   in the progression of breast cancer. In premenopausal women, estrogen (17beta-estradiol) is produced predominantly (although not exclusively) in the ovaries through aromatization of estrogen precursors (such as androstenedione) catalyzed by the enzyme estrogen
25   synthetase (aromatase). In menopausal women the ovaries no longer produce estrogen. However, aromatization of adrenal androgen can still occur in peripheral tissues, resulting in the production of 17beta-estradiol.

Estrogens are involved in cellular proliferation and
30   the maintenance of breast tissue. The proliferative effects of estrogens are also involved in the promotion of tumor growth in breast cancer, and a number of therapeutic approaches designed to reduce the amounts of

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estrogen have been developed.

In premenopausal women, removal of the ovaries (by
oophorectomy, radiation therapy, or biochemical
castration) reduces the amount of estrogen and thereby
5   reduces the proliferative effects of estrogen on tumour
growth. In women without functional ovaries,
antiestrogens and aromatase inhibitors can be used to
reduce the amount of estrogen that is produced in the
peripheral tissues.
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Anti-Hormonal (Anti-Estrogenic Steroid) Therapy
An antihormonal therapeutic agent is a chemical
agent which reduces the level of estrogen in the body, or
15   which antagonizes estrogen activity. The term "chemical
agent" is used to exclude radiation therapy or surgery.
Of course, these treatments may be used in addition to
the contemplated chemical treatment. The chemical agent
may be a biochemical, such as an enzyme or hormone. (Note
20   that some hormones inhibit the actions of other
hormones.)

A chemical agent can reduce the level of estrogen in
an organ or tissue by inhibiting the synthesis or
secretion by estrogen-roducing cells, or the transport of
25   estrogen to the organ or tissue of interest. Or it can
promote the catabolism of estrogen. A chemical agent can
also be used to reduce estrogen activity, e.g., by
inhibiting the binding of estrogen to a receptor
(pharmacological antagonism), or by inhibiting a
30   downstream activity resulting from activation of an
estrogen receptor (physiological antagonism).

Treatment with antiestrogens and/or aromatase

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inhibitors can be referred to either as "hormonal
therapy" or as "antihormonal therapy", although the
latter term is preferred. Reference to anti-hormonal
therapy, unless otherwise specified, should be taken as
5   referring to anti-estrogenic steroid therapy.

        Hormonal-therapy· and antihormonal-therapy should not
be confused with "hormone replacement therapy" (HRT)
which is a controversial medical treatment for women with
symptoms (hot flashes, night sweats etc.) associated with
10   menopause.

        For the purpose of the present claims, the term
"anti-hormonal therapy" includes (but is not limited to)
treatment with antiestrogens, aromatase inhibitors and/or
functionally equivalent compounds as, by way of example,
15   described herein below.

        Antiestrogens. Antiestrogens are used as a therapy
in the treatment of metastatic breast cancer and inhibit
estrogen-induced proliferation through interaction with
20   the estrogen receptor. One mechanism is by competitive
inhibition of the estrogen receptor, and hence the
"antiestrogens" include estrogen receptor antagonists and
SERMs (see below). It has also been suggested that
antiestrogen/estrogen-receptor complex can inhibit
25   transcription of genes that are under control of the
estrogen-response elements. As a result of this reduced
gene transcription, cell division is also reduced.

        Examples of non-steroidal antiestrogens include
toremifene, tamoxifen, droloxifene and trioxifene.
30   Fulvestrant is an example of a steroidal antiestrogen.

        Estrogen receptor antagonists. The anti-estrogenic
steroid compound may be an estrogen receptor antagonist.

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That is, it competitively inhibits the binding of
estrogen to at least one estrogen receptor, without
significantly activating the receptor in its own right.
ICI182,780 is the best known pure antagonist.

5   A large number of peptides which bind and
selectively inhibit an estrogen receptor have been
identified by combinatorial library techniques, i.e., by
screening a combinatorial library for those which bind a
particular estrogen receptor target. See Norris JD,

10   Paige LA, Christensen DJ, et al.: Peptide antagonists of
the human estrogen receptor. Science 1999, 285:744-746.
Based on knowledge of these peptides, peptidomimetics can
be designed.

15   *Selective Estrogen Receptor Modulators (SERMs).*
SERMs are compounds which competitively inhibit the
binding of estrogen to one or more of the estrogen
receptors. There are several different estrogen
receptors, and the SERMs vary with respect to their

20   spectrum of affinity for the receptors.
Unlike a pure estrogen receptor antagonist, the
SERMs activate one or more of the estrogen receptors to
which they bind. In other words, they are agonists with
respect to at least one estrogen receptor, and

25   antagonists with respect to at least one other estrogen
receptor. Consequently, they have some estrogen-like
activity, but their spectrum of activation is different
from that of the human estrogens. In other words, SERMs
are "designer" estrogens. Clearly, the SERMs of interest

30   are those whose estrogen-like activities do not include
the activities which are cancer growth-promoting.
Since the estrogen receptors vary from tissue to
tissue, the effects of SERMs may also vary from one

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tissue to the next. We are concerned with the effect of
SERMs on breast cancer tissue, as well as with any side
effects, desirable or undesirable, in any tissue.
Preferably, the SERM prevents bone loss (like estrogen)
5   and lowers serum cholesterol (like estrogen). SERMs of
interest include tamoxifen (most preferred), toremifene,
droloxifen, clomifene, arzoxifene, raloxifene, the
raloxifene analog LY 117018 and SERM EM-652.

It should be noted that a compound, especially a
10   steroid, which initially appears to be a pure estrogen
receptor antagonist may prove on closer examination to
act as an agonist for some estrogen receptors, in which
case it is reclassified as a SERM. Compounds noted here
as being as interest as estrogen receptor antagonists
15   remain of interest even if they are so reclassified.

Just as combinatorial libraries can be used to
identify peptides which act as pure estrogen receptor
antagonists, they can be used to identify SERMs. All
that is necessary is that peptides which bind one
20   estrogen receptor be screened against additional estrogen
receptors, and the peptides which bind a plurality of
receptors are then screened for agonist/antagonist
activity. One successful peptide SERMs are identified,
peptidomimetic SERMs can be designed.

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        Estrogen receptor disruptors. Drugs may inhibit the
synthesis of, permanently inactivate, or even destroy
estrogen receptors. Fulvestrant has this activity.

30   *Aromatase inhibitors*. Aromatase inhibitors reduce
the levels of estrogens by inhibiting the aromatase
enzyme complex, which is responsible for synthesizing
estrogen. Examples of aromatase inhibitors include

aminoglutethimide*, anastrozole**, vorozole, letrozole**,
liarozole, megastrole, exemestane*, and formestane* (*
is preferred, ** is most preferred). Naturally,
combinatorial libraries can be used to identify peptides
which bind to aromatase, and these peptides further
screened for inhibitory activity.

While in the discussions above, we have referred to
combinatorial peptide libraries, it should be noted that
these teachings apply, mutatis mutandis, to the screening
of other combinatorial libraries for active compounds,
using estrogen receptors or aromatase as the binding
target.

Miscellaneous Anti-Estrogenic Steroid Agents. There
are, of course, other compounds whose mechanisms of
action as less well elucidated, yet can be considered
anti-hormonal therapy. Such compounds can include
geoselin acetate (Zoladex®) and megestrol acetate
(Megase®).

The Immune System.

The ability of vertebrates to protect themselves
against infectious microbes, toxins, viruses, or other
foreign macromolecules is referred to as immunity.
Acquired or specific immunity comprises defense
mechanisms which are induced or stimulated by exposure to
foreign substances.

The events by which the mechanisms of specific
immunity become engaged in the defense against invading
microorganisms cancer cells, etc. are termed immune
responses. Vertebrates have two basic immune responses:
humoral and cellular. Humoral immunity is provided by B

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lymphocytes, which, after proliferation and
differentiation, produce antibodies which circulate in
the blood and lymphatic fluid. Cellular immunity is
provided by the T cells of the lymphatic system. The

5   cellular immune response is particularly effective
against fungi, parasites, intracellular viral infections,
cancer cells and foreign matter, whereas the humoral
response primarily defends against the extracellular
phases of bacterial and viral infections.

10   An "antigen" is a foreign substance which is
recognized (specifically bound) by an antibody or a T-
cell receptor, regardless of whether it can induce an
immune response. Foreign substances inducing specific
immunity are termed "immunizing antigens", or

15   "immunogens". An "hapten" is an antigen which cannot, by
itself, elicit an immune response (though a conjugate of
several molecules of the hapten, or of the hapten to a
macromolecular carrier, might do so). Since the present
application is concerned with eliciting immune response,

20   the term "antigen" will refer to immunizing antigens
unless otherwise stated.

Immunological Agents

An immunological agent is one which contributes to
25   acquired immunity, actively or passively, and hence
comprises at least one of the following: an immunogen, an
antibody, or an antigen-activated T cell.

Anti-Breast Cancer Immunogen
30   The immunogen of the present invention is a molecule
comprising at least one breast cancer-associated B or T
cell epitope, as defined below, and which, when suitably
administered to a subject (which, in some cases, may mean

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associated with a liposome or with an antigen-presenting cell), elicits a humoral and/or cellular immune response which is protective, at least as part of the contemplated combination therapy, against breast cancer.

Breast Cancer-Associated Epitope

The epitopes of the present invention may be directly or indirectly associated with breast cancer, with the former being preferred.

An epitope may be said to be directly associated with breast cancer if it is presented by an intracellular, surface or secreted antigen of breast cancer. It need not be presented by all breast cancer cell lines, or by all cells of a particular tumor, or throughout the entire life of the tumor. It need not be specific to the tumor in question.

An epitope may be indirectly associated with breast cancer if the epitope is of an antigen which is specifically produced or overproduced by non-breast tumor cells of the subject in specific, but non-immunological, response to the disease, e.g., an angiogenic factor which is overexpressed by nearby cells as a result of regulatory substances secreted by a tumor.

The term "breast cancer associated epitope" also includes any non-naturally occurring epitope which is sufficiently similar to an epitope naturally associated with the breast cancer so that antibodies or T cells which recognize the natural epitope also recognize the similar non-natural epitope.

Preferably, the epitope is not merely associated with breast cancer, but is specific to breast cancer. An epitope may be said to be specific to breast cancer, if it is associated more frequently with that source than

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with other sources, to a detectable and clinically useful
extent. Absolute specificity is not required, provided
that a useful prophylactic, therapeutic or diagnostic
effect is still obtained.

5   A breast cancer specific epitope is more frequently
associated with that tumor that with other tumors, or
with normal cells. Preferably, there should be a
statistically significant (p=0.05) difference between its
frequency of occurrence in association with breast

10   cancer, and its frequency of occurrence in association
with (a) normal breast cells, and (b) at least one other
type of tumor.

The term "breast cancer specific epitope" also
includes any non-naturally occurring epitope which is

15   sufficiently similar to a naturally occurring epitope
specific to breast cancer so that antibodies or T cells
stimulated by the similar epitope will be essentially as
specific as CTLs stimulated by the natural epitope.

In general, tumor-versus-normal specificity is more

20   important than tumor-versus-tumor specificity as
(depending on the route of administration and the
particular normal tissue affected), higher specificity
generally leads to fewer adverse effects. Tumor-versus-
tumor specificity is more important in diagnostic as

25   opposed to therapeutic uses.

The term "breast cancer specific" is not intended to
connote absolute specificity, merely a clinically useful
difference in probability of occurrence in association
with breast cancer rather than in a matched normal

30   subject.

The epitopes of the present invention may be B-cell
or T-cell epitopes, and they may be of any chemical

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nature, including without limitation, peptides, carbohydrates, lipids, glycopeptides and glycolipids. The epitope is at least substantially the same as a naturally occurring epitope. It may be identical to a naturally occurring epitope, or a modified form of a naturally occurring epitope.

A term such as "breast cancer-associated epitope" includes both native and mutant epitopes, but the mutant epitope must be cross-reactive with a native breast cancer-associated epitope. Likewise, "MUC1 epitope", without further qualification, is intended to encompass, not only a native epitope of MUC1, but also a mutant epitope which is substantially identical to a native epitope. Such a mutant epitope must be cross-reactive with a native MUC1 epitope.

For methods of identifying naturally occurring epitopes in a natural antigen, or of predicting the B and T cell epitopes in a polypeptide antigen for which the amino acid sequence is known, or of designing a mutant epitope based on a known natural epitope, see PCT/US03/10750, filed April 9, 2003 (atty docket KOGANTY4A-PCT).

B-cell epitopes

B-cell epitopes are epitopes recognized by B-cells and by antibodies.

B-cell peptide epitopes are typically at least five amino acids, more often at least six amino acids, still more often at least seven or eight amino acids in length, and may be continuous ("linear") or discontinuous ("conformational") (the latter being formed by the folding of a protein to bring noncontiguous parts of the primary amino acid sequence into physical proximity).

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B-cell epitopes may also be carbohydrate epitopes.

T cell Epitopes

A T cell epitope is one which can stimulate or
5   enhance a cellular immune response to that epitope.
The epitope must, of course, be one amenable to
recognition by T-cell receptors so that a cellular immune
response can occur. For peptides, the T-cell epitopes
may interact with class I or class II MHC molecules. The
10   class I epitopes usually 8 to 15, more often 9-11 amino
acids in length. The class II epitopes are usually 5-24
(a 24 mer is the longest peptide which can fit in the
Class II groove), more often 8-24 amino acids. If the
immunogen is larger than these sizes, it will be
15   processed by the immune system into fragments of a size
more suitable for interaction with MHC class I or II
molecules.

The carbohydrate T-cell epitopes may be as small as
a single sugar unit (e.g., Tn). They are preferably no
20   larger than five sugars.

Many T-cell epitopes are known. Several techniques
of identifying additional T-cell epitopes are recognized
by the art. In general, these involve preparing a
molecule which potentially provides a T-cell epitope and
25   characterizing the immune response to that molecule.
Methods of characterizing the immune response are
discussed in Ser. No. PCT/US03/10750, filed April 9,
2003, atty docket KOGANTY4A-PCT.

The reference to a CTL epitope as being "restricted"
30   by a particular allele of MHC Class I molecules, such as
HLA-A1, indicates that such epitope is bound and
presented by the allelic form in question. It does not
mean that said epitope might not also be bound and

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presented by a different allelic form of MHC, such as
HLA-A2, HLA-A3, HLA-B7, or HLA-B44.

5   *Carbohydrate Epitopes*

The epitope of the present invention may be a
carbohydrate epitope. The Tn, T, sialyl Tn (STn) and
sialyl (2->6)T epitopes are particularly preferred. The
STn epitope is the most preferred.

10   The term "carbohydrate" includes monosaccharides,
oligosaccharides and polysaccharides, as well as
substances derived from the monosaccharides by reduction
of the caronyl group (alditols), by oxidation of one or
more terminal groups to carboxylicacids, or by

15   replacement of one or more hydroxy groups by a hydrogen
atom, an amino group, a thiol group, or similar
heteroatomic groups. It also include derivatives of the
foregoing.

The epitope may be part of a carbohydrate hapten.

20   Normally, a carbohydrate hapten will not be a
polysaccharide, as a polysaccharide is usually large
enough to be immunogenic in its own right. The borderline
between an oligosaccharide and a polysaccharide is not
fixed, however, we will define an oligosaccharide as

25   consisting of 2 to 20 monosaccharide (sugar) units.

The hapten may be a monosaccharide (without
glyosidic connection to another such unit) or an
oligosaccharide. If an oligosaccharide, it preferably is
not more than 10 sugar units.

30   Monosaccharides are polyhydroxy aldehydes ($H[CHOH]_n$-
CHO) or polyhydroxy ketones ($H-[CHOH]_n-CO-[CHOH]_m-H$) with
three or more carbon atoms.

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Each monosaccharide unit may be an aldose (having an
aldehydic carbonyl or potential aldehydic carbonyl group)
or a ketose (having a ketonic carbonyl or potential
ketonic carbonyl group). The monosaccharide unit further

5   may have more than one carbonyl (or potential carbonyl)
group, and hence may be a dialdose, diketose, or
aldoketose. The term "potential aldehydic carbonyl group"
refers to the hemiacetal group arising from ring closure,
and the ketonic counterpart (the hemiketal structure).

10   . The monosaccharide unit may be a cyclic hemiacetal
or hemiketal. Cyclic forms with a three membered ring
are oxiroses; with four, oxetoses, with five, furanoses;
with six, pyranoses; with seven, septanoses, with eight,
octaviruses, and so forth. The locants of the positions

15   of ring closure may vary.

The monosaccharide unit may further be a deoxy sugar
(alcoholic hydroxy group replaced by hydrogen), amino
sugar (alcoholic hydroxy group replaced by amino group),
a thiosugar (alcoholic hydroxy group replaced by thiol,

20   or C=) replaced by C=S, or a ring oxygen of cyclic form
replaced by sulfur), a seleno sugar, a telluro sugar, a
(-substituted monosaccharide, an unsaturated
monosaccharide, an aza sugar (ring carbon replaced by
nitrogen), an amino sugar (ring oxygen replaced by

25   nitrogen) an alditole (carbonyl group replaced with CHOH
group), aldonic acid (aldehydic group replaced by carboxy
group), a ketoaldonic acid, a uronic acid, an aldaric
acid, and so forth.

Sialic acid, also known as N-acetyl neuraminic acid

30   (NANA), is of particular interest. It is the terminal
sugar on several tumor-associated carbohydrate epitopes.

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Mucin epitope

Numerous antigens of clinical significance bear carbohydrate determinants. One group of such antigens comprises the tumor-associated mucins (Roussel, et al.,

5   Biochimie 70, 1471, 1988).

In a preferred embodiment, the epitope is an epitope of a cancer-associated mucin. Generally, mucins are glycoproteins found in saliva, gastric juices, etc., that form viscous solutions and act as lubricants or

10   protectants on external and, internal surfaces of the body. Mucins are typically of high molecular weight (often > 1,000,000 Dalton) and extensively glycosylated (over 80%). The glycan chains of mucins are O-linked (to serine or threonine residues) and may amount to more than

15   80% of the molecular mass of the glycoprotein.

Mucins are produced by ductal epithelial cells and by tumors of the same origin, and may be secreted, or cell-bound as integral membrane proteins (Burchell, et al., Cancer Res., 47, 5476, 1987; Jerome, et al., Cancer

20   Res., 51, 2908f 1991).

Cell membrane mucins have distinct external, transmembrane, and cytoplasmic domains. They exist as flexible rods and protrude relatively great distances from the cell surface forming an important component of

25   the glycocalyx (Jentoff, 1990) and the terminal carbohydrate portions thereof are probably the first point of contact with antibodies and cells of the immune system.

Cancerous tissues produce aberrant mucins which are

30   known to be relatively less glycosylated than their normal counterparts (Hull, et al., Cancer Commun., 1, 261, 1989). Due to functional alterations of the protein glycosylation machinery in cancer cells, tumor-associated

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mucins typically contain short, incomplete glycans.

Thus, while the normal mucin associated with human milk fat globules consists primarily of the tetrasaccharide glycan, gal beta1-4 glcNAcp1-(gal beta1-3) gal NAc-alpha and its sialylated analogs (Hull, et al.), the tumor-associated Tn hapten consists only of the monosaccharide residue, alpha-2-acetamid-2-deoxy-D-galactopyranosyl, and the T-hapten of the disaccharide beta-D-galactopyranosyl-(1-3)alpha-acetamido-2-deoxy-D-galactopyranosyl.

Other haptens of tumor-associated mucins, such as the sialyl-Tn and the sialyl-(2-6)T haptens, arise from the attachment of terminal sialyl residues to the short Tn and T glycans (Hanisch, et al., Biol. Chem. Hoppe-Seyler. 370, 21, 1989; Hakormori, Adv. Cancer Res., 52:257, 1989; Torben, et al., Int. J. Cancer, 45 666, 1980; Samuel, et al., Cancer Res., 50, 4801-1990).

Because of these differences in glycosylation, the aberrant cancer-associated mucins are antigenically different from their normal cell counterpart mucins, exposing normally cryptic carbohydrate- (Hanish et al, 1989; Torben et al,1990; Samuel et al, 1990), peptide- (Burchell et al, 1987) and perhaps even glycopeptide-epitopes. Therefore, because cell surface mucins protrude, they themselves may serve as targets for immune attack (Henningson, et al., 1987; Fung, et al., 1990; Singhal, et al., 1991; Jerome et al., 1991; Oncogen, EP 268,279; Biomembrane Institute, WO89/08711; Longenecker, USP 4,971,795). Under some circumstances, cancer-associated cell membrane mucins can actually "mask" other cell surface antigens and protect cancer cells from immune attack (Codington et al, 1983; Friberg, 1972; Miller et al, 1977).

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The T and Tn antigens (Springer, Science, 224, 1198, 1984) are found in immunoreactive form on the external surface membranes of most primary carcinoma cells and their metastases (>90% of all human carcinomas). As

5   cancer markers, T and Tn permit early immunohistochemical detection and prognostication of the invasiveness of some carcinomas (Springer). The presence of the sialyl-Tn hapten on tumor tissue has been identified as an unfavorable prognostic parameter (Itzkowitz, et al.

10 Cancer, 66, 1960, 1990; Yonezawa, et al., Am. J. Clin. Pathol., 98 167, 1992).

The altered glycan determinants displayed by the cancer associated mucins are recognized as non-self or foreign by the patient's immune system (Springer).

15 Indeed, in most patients, a strong autoimmune response to the T hapten is observed. These responses can readily be measured, and they permit the detection of carcinomas with greater sensitivity and specificity, earlier than has previously been possible. Finally, the extent of

20 expression of T and Tn often correlates with the degree of differentiation of carcinomas (Springer).

The mucin epitope may be a core peptide, a carbohydrate, or a glycopeptide (Consequently, there is

25 overlap between the mucin epitope and carbohydrate epitope embodiments of the invention.) Non-limiting examples of mucins which may carry epitopes are the human tumor associated Thomsen-Friedenreich antigen, (MacLean, 1992), epiglycanin-related glycoprotein

30 (Codington, 1984) ovine submaillary mucin, bovine submaxillary mucin, breast tumor mucins (e.g., human polymorphic epithelial mucin, including breast tumor mucins, Gendler, 1988, 1990; breast cancer epithelial

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tumor antigen, Hareuveni, 1990, breast carcinoma, Hull,
1989), mammary tumor mucins (e.g., such as murine mammary
adenocarcinoma, Fung, 1990) carcinoma mucins such as
mucins arising from the kidney (e.g., renal cell

5   carcinoma), ovary (e.g., ovarian carcinoma-associated
sebaceous gland antigen, Layton, 1990), bladder, colon
(e.g., Sialosyl-Tn in colorectal cancer, Itzkowitz, 1990)
pancreatic tumor mucin (Lan, 1990), gallbladder, bladder,
colon (e.g., malignant colon mucosa mucins, Torbin, 1980)

10   and some lung tissues, melanoma mucins (e.g., melanoma-
associated antigen, Kahn, 1991) epithelial tumor cell
mucins, leukemia associated mucins, carcinoembryonic
antigen, or any other mucin associated with abnormal
cells according to known characteristics of cancer

15   associated mucins or abnormal mucins, such as aberrant
glycosylation (Hakomori, 1989, and Singhal, 1990).

MUC1 epitopes

       The human MUC1 gene product has been referred to by

20   various names, including MAM6, milk mucin; human milk fat
globule antigen (HMFG); human mammary epithelial antigen,
CA 15-3, CA 27.29; episialin; and polymorphic epithelial
mucin (PEM) (reviewed in Taylor-Papadimitriou et al,
1988)(for complete cites to the incompletely cited

25   references in this section, see Longenecker, et al.,
08/229,606). This mucin is strongly expressed on human
breast (Gendler et al, 1988), pancreatic (Lan et al,
1990) and certain ovarian cancer cells (Layton et al,
1990). Although the MUC1 encoded mucins expressed on

30   various cancers contain the same tandem repeat core
peptide sequence, glycosylation differences do exist
(Gendler et al, 1988; Lan et al, 1990). Because of
under-glycosylation in cancer cells, MUC-1 molecules on

25

cancer cells express cryptic epitopes which are not
expressed (i.e, are cryptic) on normal epithelial cells.

    MUC1 is the first cancer-associated mucin gene to be
cloned and mapped (Gendler et al, 1990), and has recently
5   been transfected into a murine mammary cell line, 410.4
(Lalani et al, 1991). MUC1 transfected 410.4 cells
express the MUC1 gene product on the cell surface.

    The pattern of glycosylation is similar to, but
different from, malignant cell derived mucins expressing
10 the same cryptic peptide epitopes as expressed by human
cancer associated MUC1 (Taylor-Papadimitriou et al,
1988). Lalani and co-workers (1991) have examined the
immunogenicity of the 410.4 transfectants in mice. These
workers demonstrated that mice which rejected a low dose
15 of transfected 410.4 cells did not develop tumors after a
subsequent transplant of a high dose of transfected 410.4
cells although no effect on tumor development of
untransfected wild type 410.4 cells was seen (Taylor-
Papadimitriou et al, 1988). (For complete cites, see
20 PCT/US95/04540, filed April 12, 1995 (atty docket
LONGENECKER5-PCT), and see also refs 4-11 thereof).

    It has been shown that cancer vaccines composed of
synthetic peptide antigens which mimic cryptic MUC-1
peptide sequences on cancer cells are able to induce
25 effective anti-cancer immunotherapy against MUC-1
expressing tumor cells in a murine model· Finn and co-
workers have shown that cancer patients are able to
produce specific non-MHC restricted cytotoxic T-
lymphocytes (CTL) which recognize peptide epitopes
30 expressed on MUC-1 molecules on cancer cells. (See refs.
12 and 53-55 of Longenecker5-PCT). Indeed the MUC1
sequence SAPDTRP (AAs 4-10 of SEQ ID NO:1) has been shown
to be both a T-and a B- cell epitope. It has been

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demonstrated that the immunization of chimpanzees with
synthetic MUC-1 antigens induces the development of
specific antibodies and CMI against MUC-1.

5   The human epithelial mucin MUC1 is over-expressed in
more than 90% of carcinomas of the breast, ovary and
pancreas, and in those tumors it is aberrantly
glycosylated. The SM3 antibody binds the core protein of
MUC1; it also binds the tumor glycoproteins, presumably
10   because the SM3 epitope is exposed as a result of the
aforementioned aberrant glycosylation.

The amino acid sequence of Human MUC1 is available
in the SWISS-PROT database as P15941. The number of
repeats is highly polymorphic. It varies from 21 to 125
15   in the northern european population. The most frequent
alleles contains 41 and 85 repeats. The tandemly
repeated icosapeptide underlies polymorphism at three
positions, as shown by brackets:
PAPGSTAP[P/A/Q/T]AHGVTSAP[D/E][T/S]R (SEQ ID NO:2). The
20   common polymorphisms are the coordinated double mutation
DT -> ES and the single replacements P -> A, P -> Q and
P-> T. The most frequent replacement DT > ES occurs in up
to 50% of the repeats. For Mouse MUC1, see SWISS-PROT
Q02496.

25   Moller, et al., Eur. J. Biochem. 269:1444-55 (Mar.
2002) has used NMR spectroscopy to study the binding of
the SM3 antibody to the pentapeptide MUC1 epitope PDTRP
and to the related glycopentapeptide in which the
threonine is O-lined to alpha-d-GalNAc. Moller found
30   that the PDT interacted with the SM3 antibody more
strongly than did the RP, suggesting that the RP would be
more tolerant of mutation. In contrast, the glycopeptide
interacted with SM3 using all of its amino acids,

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although the strongest effect was with the Pro1. Docking
studies were conducted; these could be performed with
mutant peptides for which 3D structures are deducible or
determined.

5   Hiltbold, et al., Cancer Res., 58:5066-70 (1998)
showed that CD4+ T-cells primed in vitro with a synthetic
MUC1 peptide of 100 amino acids, representing five
unglycosylated tandem repeats, and presented by dendritic
cells, produced IFN-gamma and had moderate cytolytic
10   activity. They also identified a core peptide sequence,
PGSTAPPAHGVT (SEQ ID NO:3), which elicits this response
when it is presented by HLA-DR3.

        Heukamp, et al., Int. J. Cancer, 91:385-92 (2001)
eleicted peptide-specific CTL immunity in A2/K(b)
15   transgenic mice with three MUC1-derived peptides that map
outside the variable number tandem repeat region. These
peptides were MUC(79-87)(TLAPATEPA)(SEQ ID NO:4),
MUC(167-175)(ALGSTAPPV)(SEQ ID NO:5) and MUC(264-
72)(FLSFHISNL)(SEQ ID NO:6). All comply with the peptide
20   binding motif for HLA-A*0201.

        Engelmann, et al., J. Biol. Chem. 276:27764-9 (Jul.
2001) report that there are three sequence variants in
the tandem repeat region of MUC1. Variant 1 replaced DT
with ES.

25   Soares et al., J. Immunol. 166: 6555-63 (Jun. 2001)
used a seven tandem repeat MUC1 peptide to elicit an
immune response. If the peptide was delivered on
dendritic cells, it only elicited T cell immunity. If
injected together with soluble peptide, Ab production was
30   also triggered.

        Von Mensdorff-Pouilly et al., J. Clin. Oncol.
18:574-83 (Feb. 2000) used a MUC1 triple tandem repeat
peptide conjugated to BSA in an immunoassay of anti-MUC1

28

antibody levels in breast cancer patients.

Denton, et al., Pept. Res. 7:258-64 (Sept./Oct.
1994), colinearly liked a MUC1 mucin B cell peptide
epitope to a known murine T cell epitope in both T-B and
5   B-T orientations. Brossart et al., Blood, 93:4309-17
(June 1999) analyzed the MUC1 amino acid sequence and
identified two novel peptides with a high binding
probability to the HLA-A2 molecule. One was from the
variable tandem repeat region, and the other from outside
10 it.

Carmon, et al., Int. J. Cancer, 85:391-7 (Feb. 2000)
evaluated the anti-tumor potential of HLA-A2.1 motif-
selected peptides from non-tandem repeat regions of the
molecule. See also Pietersz et al., Vaccine, 18:2059-71
15 (Apr. 2000).

Keil, et al. Angew. Chem. Int. Ed. Engl. 40:366-
9(Jan. 2001) conjugated a MUC1 epitope to a tetanus toxin
epitope.

Von Mensdorff-Pouilly et al., Int. J. Cancer,
20 86:702-12 (Jun. 2000) reported that the most frequent
minimal epitopic sequences of natural MUC1 IgG and IgM
antibodies were RPAPGS (AAs 9-14 of SEQ ID NO:1), PPAHGVT
(AAs 17-20 followed by AAs 1-3 of SEQ ID NO:1) and PDTRP
(AAs 6-10 of SEQ ID NO:1). MUC1 peptide vaccination
25 induced high titers of IgM and IgG antibodies
predominantly directed, respectively, to the PDTRPAP (AAs
6-12 of SEQ ID NO:1) and the STAPPAHGV (AAs 14-20
followed by AAs 1-2 of SEQ ID NO:1) sequences of the
tandem repeat. Natural MUC Abs from breast cancer
30 patients reacted more strongly with GalNac-glycosylated
peptides than with unglycosylated peptides.
See also EP Appl 1,182,210; Sandrin, USP 6,344,203; Finn,
USP 5,744,144.

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See also, Petrakou, et al., "Epitope Mapping of
Anti-MUC1 Mucin protein Core Monoclonal Antibodies" (21-
29); Imai, et al., "Epitope Characterization of MUC1
Antibodies" (30-34), Schol, et al., "Epitope
5   Fingerprinting Using Overlapping 20-mer peptides of the
MUC1 Tandem repeat sequence" (35-45), and Blockzjil,
"Epitope characterization of MUC1 Antibodies" (46-56),
all in ISOBM TD-4 International Workshop on Monoclonal
Antibodies against MUC1 Nov. 1996), reprinted in Tumor
10   Biology, 19 Suppl. 1: 1-152 (1998).

See also Von Mensdorff-Pouilly, et al., "Human MUC1
mucin: a multifacted glycoprotein," Int J. Biol. Markers,
15:343-56 (2000).

15   In some embodiments, the present invention therefore
contemplates immunogens which comprise at least one
native B and/ or T cell epitope of MUC1, or at least one
mutant epitope substantially identical to such a native
epitope. It may further comprise additional MUC1 sequence
20   which is not part of an epitope.

The immunogen may comprise both a B cell epitope and
a T cell epitope of MUC1 (which, in each case, may be a
natural epitope or an allowed mutant thereof), and these
epitopes may be identical, overlapping, or distinct.   T
25   and B cell epitopes of an antigen may overlap. For
example, in the case of MUC-1, SAPDTRP (AAs 4-10 of SEQ
ID NO:1) is a T-cell epitope, while PDTRP (AAs 6-10 of
SEQ ID NO:1) is merely a B-cell epitope.

It may further comprise additional B cell epitopes,
30   and/or additional T cell epitopes. The B cell epitopes
may be the same or different, and likewise the T cell
epitopes may be the same or different.

If the immunogen of the present invention comprises

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a MUC1-related sequence at least substantially identical
to a MUC1 sequence of at least five amino acids, the
MUC1-related sequence may comprise one or more
glycosylation sites found in the corresponding MUC1

5   sequence. It may differ from the corresponding MUC1
sequence in the number of potential glycosylation sites,
as a result of mutation, or it may have the same number
of potential glycosylation sites.

The potential glycosylation sites may be (1) sites

10   actually glycosylated in the MUC1-derived tumor
glycoprotein, (2) sites potentially glycosylatable but
not actually glycosylated in that tumor glycoprotein,
and/or (3) sites foreign to said glycoprotein. Likewise,
the actual glycosylation sites may be (1) sites actually

15   glycosylated in the MUC1-derived tumor glycoprotein, (2)
sites potentially glycosylatable but not actually
glycosylated in that tumor glycoprotein, and/or (3) sites
foreign to said glycoprotein. None, one, some or all of
the glycosylation sites normally glycosylated in the

20   MUC1-derived tumor glycoprotein may be glycosylated in
the immunogen of the present invention.

MUC1 is a polymorphic antigen characterized by a
variable number (typically 21-125, especially 41 or 85)
of perfect and imperfect repeats of the following

25   sequence:

GVTSAPDTRPAPGSTAPPAH (SEQ ID NO:1)

Since there are multiple repeats of this sequence,

30   the starting point shown is arbitrary, and an epitope may
bridge two repeats.

Consequently, the immunogens of the present
invention may comprise the aforementioned complete repeat

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sequence or a cyclic permutation thereof. Moreover, they
may comprise two or more copies of the aforementioned
repeat or a cyclic permutation thereof. Thus, in
compounds 1a and 1b, there are two copies of a cyclic

5   permutation (starting at TSA... and ending with HGV) of
the above sequence, followed by the unrelated SSL
sequence.

Each MUC1 epitope in question may correspond to an
epitope of the variable tandem repeat region, or to an

10   epitope outside that region. The former include RPAPGS
(AAs 9-14 of SEQ ID NO:1), PPAHGVT (AAs 4-10 of SEQ ID
NO:7) and PDTRP (AAs 6-10 of SEQ ID NO:1). The sequence
PDTRPAPGS (AAs 6-14 of SEQ ID NO:1) is of particular
interest, as it includes two overlapping epitopes. The

15   PDTRP sequence forms the tip of a protruding knob exposed
to solvents and forming a stable type II beta-turn.

The non- VNTR region epitopes include MUC(79-
87)(TLAPATEPA)(SEQ ID NO:7), MUC(167-175)(ALGSTAPPV)(SEQ
ID NO:8) and MUC(264-72)(FLSFHISNL)(SEQ ID NO:9).

20   Preferably, the immunogen comprises the polymorphic
epitope P[D/E][T/S]RP or a substantially identical mutant
thereof. ("Substantially identical" is defined in
Koganty4A-PCT, PCT/US03/10750, filed April 9, 2003.)
More preferably it comprises PDTRP or a substantially

25   identical mutant thereof.

In some embodiments, the immunogen comprises at
least one 20 amino acid sequence (an effective tandem
repeat) which differs solely by one or more conservative
substitutions and/or a single nonconservative

30   substitution from a tandem repeat of MUC1, and comprises
an epitope of the variable tandem repeat region of MUC1
(either identically, or an allowed mutant).

(Conservative substitutions are defined in Koganty4A-PCT,

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PCT/US03/10750, filed April 9, 2003) Preferably, it
differs solely, if at all, by conservative substitutions,
more preferably, by no more than a single conservative
substitutions, and most preferably, is identical to such
5   a tandem repeat. It should be noted that the tandem
repeats of MUC1 are imperfect and hence the sequence
could be identical to one repeat but not to another.
Also, there are allelic variations in these repeats, and
so the sequence could be identical to the sequence for
10   one allele and not for another.

In a subset of these embodiments, the immunogen
comprises a plurality of nonoverlapping effective tandem
repeats, such as two (for a total of 40 amino acids),
three (for a total of 60 amino acids), four, five, six,
15   seven or eight. These effective tandem repeats may, but
need not be, identical to each other. (In contrast, note
that in the natural human MUC1 mucin, the number of
repeats is typically 21-125.)

Besides one or more effective tandem repeats, the
20   peptide portion of the immunogen may comprise additional
amino acid subsequences. If so, these subsequences may
comprise additional epitopes, which may be MUC1 variable
tandem repeat region epitopes (falling short of a
effective tandem repeat), MUC1 epitopes from outside that
25   region, or epitopes of other cancer antigens. It may also
include an immunomodulatory element, see Longenecker5-
PCT, PCT/US95/04540, filed April 12, 1995.

Preferably, one or more of the serines and/or
threonines of the MUC1 tandem repeat are glycosylated,
30   preferably with Tn or sialyl Tn. In the natural human
MUC1 mucin, there are five normal glycosylation sites per
repeat. In normal MUC1, an average of 2.6 of these five
sites are in fact occupied. The average number of

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glycosylated amino acids per repeat may be less than, the
same as, or greater than the "natural" value.

Other Breast Cancer Associated Epitopes

5   At least one immunogen may comprise at least one
epitope of another breast cancer-associated antigen,
including but not limited to:

MAGE family, e.g. MAGE-1,2,3, and 6

10   NY-ESO-1

HER-2

P53

Kinesin 2

NY-BR-1

15   Mucins, e.g. MUC-1, and 5

Telomerase

CYFRA 21-1 (cytokeratin fragment 19)

ART 1

CEA

20   SSX family, e.g. SSX-1,2, and 4

SCP-1 (synaptonemal complex protein 1)

Clustered Epitopes

25   If the immunogen comprises a plurality of epitopes,
they may be clustered or unclustered. A cluster is here
defined as a moiety consisting of at least two directly
adjacent epitopes. The cluster may of course include
more than two epitopes in direct sequence. See, e.g.,

30   Reddish, et al., Glycoconjugate J., 14:549-60 (1997)
(clustered STn), Ragapathi, et al. Cancer Immunol.
Immunother. 48:1-8 (1999).

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Immunogen Design

A natural or non-natural immunogen may comprise one
or more epitopes and, if it comprises a plurality of
epitopes, they may be the same or different. The epitopes
5   may be clustered or unclustered.

Hapten-Carrier Conjugate

If the epitopes are B cell epitopes, then the
immunogen must be of sufficient size to elicit a humoral
10   immune response.

In a first preferred design, the epitope in question
is conjugated to an unrelated (non-breast cancer
associated) immunogenic carrier, such as KLH, albumin,
dextran, etc. The immunogenic carrier is a chemical
15   moiety which does not itself comprise any of the desired
epitopes but which, suitably conjugated to one or more
such epitopes, creates an immunogenic conjugate which
elicits a humoral immune response. Typically, the
conjugate will have a molecular weight of at least 5,000
20   daltons, more preferably at least 10,000 daltons. The
preferred maximum is the maximum exhibited by mucins,
e.g., about 5,000,000 daltons.

If the epitope and the carrier are both peptides,
then the conjugate may be expressed directly by
25   recombinant DNA technique, i.e., an artificial gene
encodes the entire conjugate. If so, then the number of
epitope-encoding segments within the artificial gene will
dictate the number of epitopes within a single conjugate
molecule.

30   Otherwise, the conjugate must be chemically
synthesized. Usually, the epitope and carrier are
prepared separately and then conjugated chemically. If
so, the number of epitopes conjugated to a single carrier

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moiety within a given conjugate molecule will vary from molecule to molecule. The maximum substitution ratio (# epitopes per conjugate molecule) will be determined by the number of reactive functionalities on the carrier.

5   For example, if the carrier is a protein, and the conjugation chemistry requires an amino function on the carrier, then the maximum substitution ratio will be the number of lysines, plus one (for the N-terminal).

Many workers have synthesized glycosides of the
10   carbohydrate haptens and of their sialylated analogs and have used these glycosides to conjugate the haptens to proteins or synthetic peptide carriers. The glycosides have generally included an aglycon moiety from which a highly reactive functionality can be generated without
15   altering the saccharide portion of the respective hapten glycoside. The "activated" hapten glycosides are then reacted with amino groups of the proteins or synthetic peptide carriers to form amide of Schiff base linkages. The Schiff base grouping can be stabilized by reduction
20   with a borohydride to form secondary amine linkages; the whole coupling process is then referred to as reductive amination. (Gray, Arch. Biochem. Biophys., 163, 426, 1974). For examples of these conjugates, see Lemieux, et at., USP 4,866,045; Naicker, et al., USP 4,935,503;
25   Kolar, USP 4,42_ 284; Feizi, USP 4,563,445; Koganty, USP 5,055,562; Jennings, USP 4,356,170; Roy, USP 5,034,516, Wong, USP 6,013,779.

Linear Multimer Design
30   It is possible to incorporate a sufficient number of epitopes into a linear immunogen so that a distinct immunogenic carrier moiety is unnecessary, i.e., the epitopes collectively act as an immunogenic carrier

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moiety for each other. In this design, a plurality of
epitopes, which may be the same or different, are
conjugated together, either directly, or with the aid of
short spacer moieties. Typically, this kind of immunogen

5   comprises 3-30 epitopes.

The linear immunogen in question can be prepared by
recombinant DNA techniques if the epitopes are all
peptide epitopes.

It is also possible to prepare an intermediate,

10   containing any desired peptide epitopes, by recombinant
DNA techniques, and then glycosylate the intermediate so
as to add on the desired carbohydrate epitopes.

Crosslinked Multimer Design

15   It is also possible to prepare smaller linear
multimers and then crosslink them together.

Dendrimer Design

In another design, the immunogen has a branched

20   structure, with one or more epitopes attached to each of
a plurality, and preferably to all, of the branches. For
example, the epitopes may be attached to a branched
lysine core structure. Each lysine can conjugate to
three other lysines by virtue of the normal N-terminal

25   amino group, the normal C-terminal carboxy group, and the
epsilon amino group on its side chain. It should be
noted that this dendrimer design usually differs from the
conventional hapten-carrier conjugate in that the core
(the carrier) is branched, not linear, and in that the

30   core is a relatively small portion of the entire
conjugate, e.g., less than half of the molecular weight
of the conjugate.

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This recitation of possible immunogen designs is not intended to be exhaustive.

Protein Carrier

5   The preferred protein carrier, if any, in the immunogens of the present invention is a macromolecule with, in monomeric form, a molecular weight of at least 10 kD, and which contains one or more lysine residues. Preferably, it is at least 3% lysine (by moles).

10   The preferred protein carrier is a hemocyanin, such as an arthropod or molluscan hemocyanin. Hemocyanins of gastropods, especially of the Fissurellidae (keyhole limpets), and in particular the keyhole limpet (Megathura crenulata) hemocyanin, are most preferred.

15   Hemocyanins are the oxygen transport proteins of many arthropods and molluscs. Keyhole limpet hemocyanin, in nature, is a multimer, with a total MW of about 8,000 kDa. The monomer is about 400 kDa. It consists of two immunologically and physiologically distinct isoforms,

20   KLH1 and KLH2. Both are present in the hemolymph as cylindrical didecamers. Each isoform monomer contains eight functional units (FUs), termed "a" to "h" from the N- to C-terminal. FUs "b" to "g" of KLH1 total 2141 a.a., and FUs "b" to "h" of KLH2 total 2473 a.a. See

25   Altenheim, et al. "Sequence of Keyhole Limpet Hemocyanin", Abstract, http://www.sb-roscoff.fr/IO2BiP/IO2B1PP.PDF; Swerdlow, Comp. Biochem. Biophys. 113B:537-48 (1996); Stoeva, Biochem. Biophys. Acta 1435:94-109 (1999); Harris and Markl, Micron.,

30   30:597-623 (1999). Swerdlow reports that KLH-A is 449 kDa and KLH-B is 392 kDa. Sohngen, et al., Eur. J. Biochem., 248:602-14 91997) reports that KLH1 is 400 kDa

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and KLH2 is 345 kDa. Ebert, USP 5,855,919 uses the value
of 400 kDa.

     Preferably, in the conjugates of the present
invention, if the monomeric unit of the carrier moiety is
5   the KLH monomer, the conjugate is not a substituted
decamer, didecamer or multidecamer of the KLH monomer.

     The native KLH is rich in copper, but copper is lost
during reductive amination. KLH is glycosylated, with a
carbohydrate content of about 4% of molecular mass. See
10   Harris, _supra_.

     Preferably, if KLH monomer is used, at least one
carbohydrate hapten moiety will be one not natively
associated with KLH. Preferably, at least one component
sugar of the carbohydrate hapten moieties will be one not
15   natively associated with KLH.

Aggregated Multimeric Protein Carrier

     In a preferred embodiment, the epitopes are
20   conjugated to an aggregated multimeric protein carrier,
in particular, aggregated KLH. **(Note that here the term
"multimeric" refers to the number of copies of the
carrier moiety.)**

     The aggregation results from the interaction of
25   individual monomers of the protein carrier to form a
multimeric entity. The interaction may be through
binding, and/or through entanglement of the individual
protein chains (before, during or after attachment of the
carbohydrate haptens). If binding contributes to the
30   oligomerization, it may be covalent and/or noncovalent.

     Preferably, the aggregation occurs more or less
simultaneously with the attachment of the carbohydrate
haptens to the protein.

The multimeric entities preferably are dimers, trimer, tetramers, and/or pentamers of the monomeric unit of the protein carrier.

5   It is believed that the immunogenic potency of these preparations is attributable to the combination of a high hapten substitution ratio, and the aggregation of the protein carriers to form multimeric entities.

10   Degree of Aggregation

The preferred immunogen of the present invention is an aggregated, carbohydrate hapten-substituted KLH. Each monomeric unit may be KLH1, KLH2 or some other KLH monomer.

15   The term "substituted KLH monomer", as used herein, means KLH substituted with a plurality of carbohydrate haptens in addition to those with which it is natively associated. These could be duplicates of existing native carbohydrate chains, but more usually will include

20   haptens not natively associated with KLH. The KLH may, but need not, be deglycosylated to remove some or all of the native carbohydrate, specifically or nonspecifically, before hapten substitution.

The molecular weight of the substituted KLH monomer

25   will be greater than that of the unsubstituted KLH. If the latter is 400 kDa (literature values range from 345 to 449 kDa), then the substituted KLH will be of greater MW. The increment will depend on the molecular weight of each hapten moiety (including the linker) and on the

30   number of hapten moieties per monomer.

If the unsubstituted KLH monomer is 400 kDa, then a substituted dimer necessarily has molecular weight greater than 800 kDa. Hence, the substituted aggregate

preferably has an apparent molecular weight of more than 800 kDa, more preferably more than 1,200 kDa, still more preferably more than 1,600 kDa.

KLH in the preferred carbohydrate hapten-substituted monomeric form has a molecular weight of about 500 KD; without the added carbohydrate (and linkers), it is about 400 kD. Thus, hapten substitution may increase molecular weight by 25%, or more, relative to the unsubstituted KLH monomer. It follows that the substituted aggregate also preferably has an apparent molecular weight of at least 1,000 KDa, more preferably at least 1,500 KDa, even more preferably at least 2,000 KDa.

It will be understood that the preparation may comprise a heterogeneous mixture of n-mers, e.g., monomers, dimers, trimers, tetramers, etc., so that the apparent molecular weight is actually the weighted average of the apparent molecular weight of each size class of n-mer.

The preparation could theoretically be fractionated by molecular weight to determine the fraction attributable to each size class of n-mer. Preferably, monomers are less than 50% (by weight) of the preparation, more preferably less than 25%, still more preferably less than 10%, most preferably less than 5%. The preparation may also be fractionated with the goal of discarding the predominantly monomeric fraction(s) and thereby enriching for multimers.

The maximum limit on the degree of aggregation is that the aggregate should not be so large as to precipitate out of solution. However, the apparent molecular weight is preferably less than 5,000 kDa (equivalent to a substituted decamer), and more

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preferably less than 2,500 kDa (equivalent to a
substituted pentamer).

Apparent molecular weight is preferably determined
by laser light scattering. See Wyatt, Anal. Chim. Acta,
5   272:1-40 (1993). It may be estimated by size exclusion
(molecular sieve) chromatography, as set forth in
Krantz2.1-PCT, PCT/US02/24735, filed Aug. 5, 2002.

Preferably, the aggregated immunogen of the present
10   invention has a potency which is at least 200% of that of
a conjugate of the same hapten and carrier, in the same
hapten carrier monomer substitution ratio, wherein the
carrier is unaggregated. For this purpose, potency is
measured by the antibody response of immunized mice.
15

STN-KLH Conjugates
The most preferred immunogen of the present
invention is an immunogen comprising STn, preferably as a
conjugate to KLH, and most desirably similar or identical
20   to the immunogen incorporated into Biomira's Theratope®
vaccine.
Preferably, the KLH carrier is aggregated, so that
each conjugate molecule comprises, on average, at least a
KLH dimer, more preferably a KLH trimer, and still more
25   preferably a KLH tetramer.
Preferably, the NANA content is greater than 3%,
more preferably at least 5%, such as about 7%.

The preferred immunotherapeutic compound of this
30   invention can be made according to International
Application Number PCT/US02/24735 (International
Publication Number WO03015796). See also International

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Application number PCT/US90/01856 (International
Publication Number WO9011764).

Conjugation

In the case of the chemical conjugates, one or more
hapten molecules are conjugated to each carrier molecule.
The point of attachment on the carrier is ordinarily an
accessible amino group, such as the amino terminal of the
carrier, or more usually the epsilon amino group of
lysine.

The hapten is conjugated to this point of
attachment, either directly, or through a linker.
Usually, the linker is not a carbohydrate or peptide
itself. The linker, if any, is preferably a small
aliphatic group consisting of carbon, hydrogen, and
optionally, oxygen, nitrogen and/or sulfur, of not more
than 12 atoms other than hydrogen. More preferably it is
an alkyl group, linear or branched, of not more than 12
carbon atoms. Even more preferably it is $-(CH_2)_n$ where
n=1 to 12. Most preferably it is $-CH_2CH_2-$. Each linker
will connect an oxygen of the carbohydrate hapten to an
amino nitrogen, i.e., the epsilon nitrogen of lysine, or
the amino terminal of the protein carrier.

The linker may be bifunctional (attaching just one
hapten to the carrier monomer) or polyfuntional (in which
case one linker may attach a plurality of haptens to the
carrier monomer).

A "linking agent" is reacted with A and B to form
the structure A-linker-B, the "linker" being related in
structure to the original linking agent. The reaction
may be simultaneous, or the linking agent may be reacted
first with A to form the structure A-linking arm, and
then the latter with B to form A-linker-B.

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If the hapten-linking arm is hapten-crotyl (e.g.,
STn-crotyl), then ozonolysis generates a reactive hapten
aldehyde, which can be used in reductive amination of the
carrier to yield hapten-CH_2CH_2-carrier, i.e., the

5 preferred two-carbon linker. The hapten is usually O-
linked to the linker, but other linkages are possible.

Another linking agent of interest is an MMCCH
linking agent, 4-(4-maleimidomethyl)cyclohexane-1-
carboxyl hydrazide. See Ragaputhi, et al., Cancer

10 Immunol. Immunother. 48:1-8 (1999).

Some polyfunctional linkers based on crotyl linking
chemistry are depicted in Figure 1 of Reddish, et al.,
Glycoconjugate J., 14:549-60 (1997).

15 Methods of carrying out conjugation reactions and,
in particular, of preparing an Stn-KLH conjugate in which
the KLH is aggregated, are further described in
Krantz2.1-PCT, PCT/US02/24735, filed Aug. 5, 2002.

20 Hapten-Substitution Ratio

Preferably, the ratio of hapten to carrier is at
least 10 molecules of conjugated hapten to each carrier
monomer. The maximum ratio is determined by the number
of accessible attachment sites. Usually, the ratio is in

25 the range of 10-120.

In the case of sialylated conjugate, such as an
STn/KLH conjugate, the NANA content is indicative of the
hapten substitution ratio (the number of sialylated
haptens per carrier monomer). NANA content may be

30 assayed as set forth below.

In the case of an STn/KLH conjugate, the NANA
content is preferably in excess of 3%, more preferably at
least 4%, still more preferably at least 5%, even more

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preferably at least 6%, most preferably at least 7%.
Preferred values include those values in excess of 3%
which are set forth in Tables 1 and 2.

5   The maximum possible NANA content is a function of
the total number of possible STn attachment sites on the
KLH. Assuming that an STn is attached to every lysine
side chain of KLH, the NANA content would be about 12% by
weight of conjugate. This does not include the molecular
weight of the linker or the Tn. If calculated relative
10   to the molecular weight of the unsubstituted KLH, it
would be about 13%. The whole hapten-linker arm content
would be about 19% relative to the molecular weight of
the unsubstituted KLH.

Higher NANA content is primarily achieved by
15   increasing the ratio of hapten-to-KLH in the
glycosylation reaction.

If the amount of hapten is increased to elevate NANA
content, but this does not result in an increase in
immunogenicity, then the extra hapten is, in effect,
20   wasted. Hence, it may be desirable to limit the NANA
content, for economic reasons, to be not more than 10% by
weight.

Thus, the NANA content of an STn-KLH conjugate is
most preferably in the range of 6 to 10% by weight.

25

Immunomodulators

The immunogens of the present invention, which
comprise breast cancer associated epitopes, may be used
in conjunction with substances which, while not such
30   immunogens, modulate the immune response to such
immunogens in a desirable manner.

Adjuvants

45

The immunogens of the present invention may be used in conjunction with any known adjuvant. The adjuvant may be chemical or microbial in nature. Possible adjuvants include Enhanzyn™, Lipid-A, CFA, SAF-1, MDPI BCG,

5   liposomes, and Bordetella pertussis toxin, and their derivatives and analogues. Enhanzyn™ is preferred, but the present invention is not limited to the use of any particular adjuvant. The tumor-associated hapten may be conjugated to other carrier proteins, such as tetanus or

10   diphtheria toxoid, or retrovirus peptides (e.g., VP6 viral peptide),, rather than KLH, and the hapten/molecule-to-carrier molecule substitution ratio may be varied. Either natural, or synthetic antigens which cross-react with the immunosuppressive mucin may be

15   employed.

       Cyclophosphamide

       The immunogens of the present invention may be used in conjunction with an immunopotentiating amount of

20   cyclophosphamide. Cyclophosphamide (N, N-bis[2-cholorethyl]tetrahydro-2H-1,3,2-oxazaphosphorine-2-amine-2-oxide), a nitrogen mustard derivative, is a cytotoxic agent which causes cross-linking of DNA. It is most effective against rapidly dividing cells, hence its use

25   in cancer chemotherapy. Since it also destroys lymphocyte cells, it is also useful as a immunosuppressive agent, indeed, it is one of the most potent immunodepressants known.

30   Although most chemotherapeutic agents suppress host immunity, it has been demonstrated that certain chemotherapeutic agents, under specific conditions, are able to augment host anti-tumor immunity. It is believed

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that this can arise because some tumors express mucins
which elicit suppressor T cell activity, and it is that
response whose inhibition is desirable.

The time interval between administration of the
5   cyclophosphamide and administration of the synthetic
tumor-associated glycoconjugate is not fixed, but is
dependent on the time of onset and duration of action of
the cyclophosphamide's inhibitory effect on suppressor T
cell activity or on the induction of such activity by
10 tumor-expressed mucins. The dosage of cyclophosphamide
may be selected to increase the antigenic specificity of
the anti-suppressor T cell activity effect. ·

In place of cyclophosphamide, another antagonist of
15 immunosuppression may be employed,, such as other
oxazaphosphorines, cimetidine or an anti-(suppressor
cell) or anti-(suppressor factor) monoclonal antibody.
Numerous antibodies of these two types are offered for
sale (see Linscott's Directory of Immunological and
20 Biological Reagents, p. 10, 5th ed., 1988,89).

The present invention is not to be restricted on the
basis of the present interpretation of the mechanism
whereby cyclophosphamide or a similar agent exercises an
25 immunopotentiating effect. An agent antagonizes the
immunosuppressive effect of a tumor-associated mucin if
it interacts with the mucin or the T cell so that the
mucin no longer activates suppressor T cell activity, or
if it interacts with a T cell so activated or its
30 suppressor factors so as to diminish the suppressor
activity induced by said mucin, or if it interacts with
other components of the cellular immune system so as to
render them less vulnerable to suppressor T cells

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activated by said mucin or to suppressor factors released
by such cells, in another embodiment, a monoclonal
antibody specific for an epitope of a tumor-associated,
immunosuppressive mucin is attached to a suitable support
5   to form an immunosorbent. Circulating tumor-associable
immunosuppressive mucins recognized by the immunosorbent
are removed from the patient's bloodstream by
plasmapheresis. The immune response to the tumor, with or
without further stimulating the immune system by active
10   specific tumor immunotherapy, is thereby enhanced.
(Lectins or other binding substances might be-used in
place of antibodies).

Progestins and Anti-progestins
15   ·Progestins (such as progesterone) are another type
of hormone that play a role in the normal development of
breast tissue. The evidence as to the effect of
progrestins on the development of breast cancer is mixed.
Both progestins and anti-progestins have been proposed
20   for use in the treatment of breast cancer. To the extent
that administration of a progestin or an anti-progestin
can have a protective effect against breast cancer, its
use is within the contemplation of the present invention.
Mifepristone (RU 486) is an example of an anti-progestin.
25

Chemotherapy
In addition to anti-hormonal (anti-estrogenic
steroid) therapy and immunotherapy, other chemotherapy
30   may be employed. Anthracyclines (for example:
doxorubicin, daunorubicin, epirubicin, idarubicin) may be
used in the treatment of metastatic breast cancer.
Several mechanisms of action may play a role in the anti-

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tumour effects of anthracyclins, and include:
intercalation of DNA, interaction with topoisomerase II,
causing strand breaks (single and double) in DNA and
formation of free radicals.

5

Taxanes (for example: paclitaxel, docetaxel) may also be
used in the treatment of metastatic breast cancer.
Taxanes are antimicrotubular compounds that inhibit cell
division by binding to tubulin and inhibiting

10   microtubular disassembly that is required for cell
division.

Use in Conjunction (Combination)
        When two therapies are used in conjunction (or

15   combination), it means that both therapies are employed,
but it does not mean that they necessarily are
administered simultaneously. Rather, it means that one
therapy is used while the other therapy is still
exercising an effect on the patient. Preferably, they are

20   used at such times as to have a synergistic effect, that
is, the combined effects are greater than those which
would be reasonably expected as the simple additive
effect of the individual therapies.

25   *Therapeutically Effective*
        A therapy, or combination of therapies, is
therapeutically effective if there is a statistically
significant (p=0.05) improvement in the therapeutic
outcome in the treatment group as compared to a control

30   group.   It is not required that the therapy cure or even
merely alleviate the condition of all treated patients.
        When a combination therapy is contemplated, the
individual agents may be used in amounts which

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individually would not be therapeutically effective, if
they would be effective in combination.

Immunogenic Compositions

5   The immunogens of the present invention may be
administered as a component of an immunogenic
composition. Immunogenic compositions are compositions
which comprise, as at least one immunological agent, an
immunogen.

10   The immunogenic composition may further comprise a
liposome. Preferred liposomes include those identified in
Jiang,et al., PCT/US00/31281, filed Nov. 15, 2000 (our
docket JIANG3A-PCT), and Longenecker, et al., 08/229,606,
filed April 12, 1994 (our docket LONGENECKER5-USA, and

15   PCT/US95/04540, filed April 12, 1995 (our docket
LONGENECKER5-PCT). A variety of methods are available
for preparing liposomes, as described in, e.g., Szoka et
al., Ann. Rev. Biophys. Bioeng. 9:467 (1980), U.S. Patent
Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019369,

20   incorporated herein by reference.

        The composition may further comprise antigen-
presenting cells, and in this case the immunogen may be
pulsed onto the cells, prior to administration, for more

25   effective presentation.
        The composition may contain auxiliary agents or
excipients which are known in the art. See, e.g., Berkow
et al, eds., *The Merck Manual*, 15th edition, Merck and
Co., Rahway, N.J., 1987; Goodman et al., eds., *Goodman*.

30   *and Gilman's The Pharmacological Basis of Therapeutics*,
8th edition, Pergamon Press, Inc., Elmsford, N.Y.,
(1990); *Avery's Drug Treatment: Principles and Practice
of Clinical Pharmacology and Therapeutics*, 3rd edition,

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ADIS Press, LTD., Williams and Wilkins, Baltimore, MD. (1987), Katzung, ed. Basic and Clinical Pharmacology, Fifth Edition, Appleton and Lange, Norwalk, Conn. (1992), which references and references cited therein, are

5   entirely incorporated herein by reference.

       A composition may further comprise an adjuvant to nonspecifically enhance the immune response. Some adjuvants potentiate both humoral and cellular immune response, and other s are specific to one or the other.

10 Some will potentiate one and inhibit the other. The choice of adjuvant is therefore dependent on the immune response desired.

       A composition may include immunomodulators, such as cytokines which favor or inhibit either a cellular or a

15 humoral immune response, or inhibitory antibodies against such cytokines.

Pharmaceutical Purposes

20   A purpose of the invention is to protect subjects against a disease. The term "protection", as in "protection from infection or disease", as used herein, encompasses "prevention," "suppression" or "treatment." "Prevention" involves administration of a Pharmaceutical

25 composition prior to the induction of the disease. "Suppression" involves administration of the composition prior to the clinical appearance of the disease. "Treatment" involves administration of the protective composition after the appearance of the disease.

30 Treatment may be ameliorative or curative.

       It will be understood that in human and veterinary medicine, it is not always possible to distinguish between "preventing" and "suppressing" since the ultimate

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inductive event or events may be unknown, latent, or the patient is not ascertained until well after the occurrence of the event or events. Therefore, it is common to use the term "prophylaxis" as distinct from

5   "treatment" to encompass both "preventing" and "suppressing" as defined herein. The term "protection," as used herein, is meant to include "prophylaxis." See, e.g., Berker, *supra*, Goodman, *supra*, Avery, *supra* and Katzung, *supra*, which are entirely incorporated herein by

10   reference, including all references cited therein.

The "protection" provided need not be absolute, i.e., the disease need not be totally prevented or eradicated, provided that there is a statistically significant improvement (p=0.05) relative to a control

15   population. Protection may be limited to mitigating the severity or rapidity of onset of symptoms of the disease. An agent which provides protection to a lesser degree than do competitive agents may still be of value if the other agents are ineffective for a particular individual,

20   if it can be used in combination with other agents to enhance the level of protection, or if it is safer than competitive agents.

The effectiveness of a treatment can be determined by comparing the duration, severity, etc. of the disease

25   post-treatment with that in an untreated control group, preferably matched in terms of the disease stage.

The effectiveness of a prophylaxis will normally be ascertained by comparing the incidence of the disease in the treatment group with the incidence of the disease in

30   a control group, where the treatment and control groups were considered to be of equal risk, or where a correction has been made for expected differences in risk.

52

In general, prophylaxis will be rendered to those considered to be at higher risk for the disease by virtue of family history, prior personal medical history, or elevated exposure to the causative agent.

Methods of Administration

The therapeutic agents of the present invention may be administered by any effective route of administration, including oral or parenteral, the latter including intravenous, subcutaneous, and intramuscular.

Dosage Forms

The therapeutic agents of the present invention may be administered in any effective dosage form, including tablets, capsules, injectants, and so forth.

The immunogen may be delivered in a manner which enhance, e.g., delivering the antigenic material into the intracellular compartment such that the "endogenous pathway" of antigen presentation occurs. For example, the immunogen may be entrapped by a liposome (which fuses with the cell), or incorporated into the coat protein of a viral vector (which infects the cell).

Another approach, applicable when the immunogen is a peptide, is to inject naked DNA encoding the immunogen into the host, intramuscularly. The DNA is internalized and expressed.

It is also possible to prime autologous PBLs with the compositions of the present invention, confirm that the PBLs have manifested the desired response, and then administer the PBLs, or a subset thereof, to the subject.

Dosage Schedule

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The present invention contemplates the use of a combination of agents, and these may have different dosage schedules.

It is understood that the effective dosage will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The ranges of effective doses provided below are not intended to limit the invention and represent preferred dose ranges. However, the most preferred dosage will be tailored to the individual subject, as is understood and determinable by one of skill in the art, without undue experimentation. This will typically involve adjustment of a standard dose, e.g., reduction of the dose if the patient has a low body weight. See, e.g., Berkow et al, eds., *The Merck Manual*, 15th edition, Merck and Co., Rahway, N.J., 1987; Goodman et al., eds., *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th edition, Pergamon Press, Inc., Elmsford, N.Y., (1990); *Avery's Drug Treatment: Principles and Practice of Clinical Pharmacology and Therapeutics*, 3rd edition, ADIS Press, LTD., Williams and Wilkins, Baltimore, MD. (1987), Ebadi, *Pharmacology*, Little, Brown and Co., Boston, (1985); Chabner et al., *supra*; De Vita et al., *supra*; Salmon, *supra*; Schroeder et al., *supra*; Sartorelli et al., *supra*; and Katsung, *supra*, which references and references cited therein, are entirely incorporated herein by reference.

Prior to use in humans, a drug will first be evaluated for safety and efficacy in laboratory animals. In human clinical studies, one would begin with a dose expected to be safe in humans, based on the preclinical data for the drug in question, and on customary doses for

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analogous drugs (if any). If this dose is effective, the
dosage may be decreased, to determine the minimum
effective dose, if desired. If this dose is ineffective,
it will be cautiously increased, with the patients
5   monitored for signs of side effects. See, e.g., Berkow,
et al., eds., The Merck Manual, 15th edition, Merck and
Co., Rahway, N.J., 1987; Goodman, et al., eds., Goodman
and Gilman's The Pharmacological Basis of Therapeutics,
8th edition, Pergamon Press, Inc., Elmsford, N.Y.,
10   (1990); Avery's Drug Treatment: Principles and Practice
of Clinical Pharmacology and Therapeutics, 3rd edition,
ADIS Press, LTD., Williams and Wilkins, Baltimore, MD.
(1987), Ebadi, Pharmacology, Little, Brown and Co.,
Boston, (1985), which references and references cited
15   therein, are entirely incorporated herein by reference.
The total dose required for each treatment may be
administered in multiple doses (which may be the same or
different) or in a single dose, according to an
immunization schedule, which may be predetermined or ad
20   hoc. The schedule is selected so as to be
therapeutically effective, i.e., to provide protection.
The doses adequate to accomplish this are defined as
"therapeutically effective doses." (Note that a schedule
may be therapeutically effective even though an
25   individual dose, if administered by itself, would not be
effective, and the meaning of "therapeutically effective
dose" is best interpreted in the context of the
immunization schedule.)
Amounts effective for this use will depend on, e.g.,
30   the agent, the manner of administration, the stage and
severity of the disease being treated, the weight and
general state of health of the patient, and the judgment
of the prescribing physician.

Typically, the daily dose of an active ingredient of a pharmaceutical, for a 70 kg adult human, is in the range of 10 nanograms to 10 grams. For immunogens, a more typical daily dose for such a patient is in the

5 range of 10 nanograms to 10 milligrams, more likely 1 microgram to 10 milligrams. However, the invention is not limited to these dosage ranges.

The doses may be given at any intervals which are effective. In the case of immunogens, if the interval is

10 too short, immunoparalysis or other adverse effects can occur. If the interval is too long, immunity may suffer. The optimum interval may be longer if the individual doses are larger. Typical intervals are 1 week, 2 weeks, 4 weeks (or one month), 6 weeks, 8 weeks (or two months)

15 and one year. The appropriateness of administering additional doses, and of increasing or decreasing the interval, may be reevaluated on a continuing basis, in view of the patient's immunocompetence (e.g., the level of antibodies to relevant antigens).

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EXAMPLES

Background: THERATOPE® vaccine is an investigational
therapeutic cancer vaccine consisting of a synthetic form

5   of the tumor associated antigen Sialyl Tn (STn)
conjugated to the aggregated carrier protein keyhole
limpet hemocyanin (KLH), at a hapten substitution ratio
yielding a NANA content of about 7%.

Patients and Methods: Metastatic breast cancer patients

10   (MBC pts) who had no evidence of disease (NED) or non-
progressive disease (NPD) following any first-line
chemotherapy were randomized 1:1 to receive adjuvant plus
THERATOPE® vaccine or control [adjuvant plus KLH]. All
patients received a single, low-dose, IV infusion of

15   cyclophosphamide before vaccine. Primary endpoints were
time to disease progression (TDP) and overall survival
(OS). Pts were stratified by disease status and
concomitant hormone therapy (HT).

Introduction

20   An international phase III clinical trial comparing
Theratope® vaccine (STn-KLH) to control vaccine (KLH
alone) in 1028 women with stable or responding metastatic
breast cancer following any first line chemotherapy, was
conducted. The primary efficacy endpoints were survival

25   and time to disease progression (TDP). The two patient
treatment groups were well balanced in terms of
prognostic characteristics including age, estrogen
receptor (ER) status, progesterone receptor (PR) status,
HER2 expression, time from primary diagnosis to first

30   metastasis, disease burden, response to chemotherapy, and
concomitant hormone therapy use.

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While the trial yielded disappointing efficacy results in
terms of the overall intent-to-treat (ITT) population
(survival Cox p = 0.916 and TDP Cox p = 0.353),
encouraging results were observed in the **subgroup** of
patients who received THERATOPE® vaccine concomitantly
with anti-hormone (anti-estrogenic steroid) therapy. This
therapy was with SERMs and/or aromatase inhibitors, with
the precise anti-hormone therapy varying from patient to
patient. The benefit was observable for both the SERM
subpopulation and the aromatase inhibitor subpopulation.

The benefit was particularly striking for patients in
this subgroup who also developed an immune response to
THERATOPE® vaccine at or higher than the median response.
This report will discuss in detail the findings of these
exploratory analyses, as well as describe the survival
outcomes following updates.

Efficacy Outcomes by ITT Strata

The original protocol stratified patients by response to
chemotherapy into no evidence of disease (NED) or non-
progressive disease (NPD). Of the 1028 women randomized
into the trial, approximately 150 patients were enrolled
under this original protocol. In an effort to increase
the rate of enrollment, an amendment was approved at each
trial site that allowed the enrollment of women who were
receiving concomitant anti-tumor hormone therapy for
treatment of their metastatic breast cancer, an exclusion
criterion in the original protocol. To minimize the
potential for bias, it was decided to stratify patients
according to this hormone use, in addition to response to

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chemotherapy, at the time of study entry. The primary
analyses utilized the Cox Proportional Hazards model,
which included time from diagnosis to first metastasis,
as well as the two stratification variables, as
5   explanatory variables.

At the time of the final analysis, TDP and survival were
calculated for each of the strata. The results of these
calculations are shown in Table 1.

10

Both TDP and survival advantages were observed for
patients in the non-progressive disease strata who
received hormone therapy concomitantly with THERATOPE®
vaccine (n=152), compared to those in the same strata who
15   received KLH control (n=143). Additionally, patients
receiving KLH control who had no evidence of disease and
did not receive concomitant hormone therapy (n=38)
appeared to do better in terms of survival, but not TDP,
than those in the comparable THERATOPE® vaccine arm
20   (n=40). None of the differences between treatment groups
in any strata combination achieved statistical
significance.

Efficacy Outcomes by ITT Hormone Groups

25   The total numbers of patients with non-progressive
disease formed a large majority when compared to the
summation of the NED patients (916 versus 112 patients,
respectively). When the patients were examined by
hormone status, approximately one third were stratified

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to concomitant hormone therapy compared to no hormone therapy (329 versus 699 patients respectively). Although stratification by hormone use was not equally balanced compared to no hormone use, the numbers of patients in each group were large enough to justify exploratory statistical comparisons. These data are shown in Table 2.

When examining the data by hormone group, patients from either treatment group who did not receive concomitant hormone therapy were observed to have a similar median survival and TDP. However, the median survival of patients receiving THERATOPE® vaccine concomitantly with hormone therapy was 4.2 months longer than that of patients receiving KLH control concomitantly with hormone therapy. With regards to TDP, a 2.5-month benefit for THERATOPE® vaccine treated patients receiving concomitant hormone therapy was observed compared to patients in the relevant comparison group receiving KLH control. Although the benefit for THERATOPE® vaccine treated patients in the hormone subset did not reach statistical significance, the results were clinically compelling, and warranted further investigation.

Antibody Response

THERATOPE® vaccine is designed to stimulate an immune response to the tumor-associated antigen, sialyl Tn (STn). This immune response potentially results in a therapeutic benefit. As a secondary objective, immune response was included in the phase III trial. Sera from patients in this trial were assayed for IgG and IgM

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antibody responses to STn, and additionally to KLH, and Ovine Submaxillary Mucin (OSM, a naturally occurring monomeric and clustered STn).

5   Serum assays were performed at baseline, week 12 (following receipt of four adjuvanted vaccinations), week 24 and then at 3-month intervals until documented disease progression. Serum from 364 THERATOPE® vaccine treated, and 323 KLH control treated patients, was available for the week 12 assessment of antibody response. Assay

10 results by treatment arm for all patients and by hormone group (H = hormone, NH = no hormone) are shown in Table 3.

The lower response to KLH in the THERATOPE® vaccine patients may be due to the fact that STn clusters on

15 THERATOPE® vaccine block some of the epitopes on the KLH; therefore THERATOPE® vaccine treated patients had reduced exposure to KLH.

Survival and TDP by Antibody Response for All Patients

20 In phase II trials, the magnitude of the IgG anti-OSM titre was associated with improved survival [1]. Therefore, exploratory analyses were conducted to assess whether a correlation with response at week 12 and survival would be observed utilizing data from the phase

25 III trial. No statistically significant survival differences were observed in relation to IgM responses. The survival by median IgG antibody response to each of the antigens is shown in Table 4.

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For THERATOPE® vaccine treated patients, a 7.2-month
survival advantage was observed for those with an IgG OSM
titre at or greater than the median, compared to those
with a response below the median, with a trend toward
5   statistical significance. A titre at or higher than the
median to STn and KLH was also associated with improved
survival, but in neither case was the improvement
statistically meaningful. No statistically significant
differences in TDP were observed based on antibody
10   response to any of the three antigens tested.

A different pattern emerged for the KLH control patients.
Both the survival and TDP of these patients was longer
for those whose IgG anti-KLH response was less than the
15   median compared to patients with responses at or higher
than the median. The rationale for these outcomes are
not understood, however, several prognostic variables
appeared to favor those patients who developed the lower
antibody response to KLH, and may have contributed to the
20   better survival and TDP observed in this group. These
include a greater number of ER+ versus ER- women (68%
versus 59%), a greater number of superficial or bone only
versus visceral metastases (26% versus 16.6%), and a
greater number of women with normal versus elevated
25   CA 27.29 at baseline (57.1% versus 51%).

Survival by Antibody Response for the Hormone Subset

Survival by antibody response was also examined within
the subset of patients who received concomitant anti-
30   cancer hormone therapy. Although this subset had a

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better overall survival outcome compared to patients who did not receive concomitant hormone therapy while on study, the difference was particularly striking for THERATOPE® vaccine treated patients who had an anti-OSM
5   IgG response at or above the median, and for KLH control patients who had an anti-KLH IgG response below the median. The difference in survival by response to STn and KLH in the THERATOPE® vaccine treated hormone subset was not statistically significant. The data are shown in
10   Table 5.

Survival by Antibody Response for the Non-Hormone Subset

Survival by antibody response was also examined for those patients who did not receive concomitant hormone therapy. Antibody data was available from 231 THERATOPE® vaccine
15   and 206 KLH control treated patients.

For patients who received THERATOPE® vaccine without concomitant hormone therapy, no significant differences in survival were observed related to antibody response to any of the antigens tested. Median survival by a-OSM
20   response was 25.1 months for patients with responses at or above median and 23.7 for patients with responses below median (Cox p=0.8065); median survival by a-STn response was 24.0 months for patients with responses at or above median, and 24.3 for those with responses below
25   median (Cox p=0.9347); median survival by a-KLH response was 26.1 months for patients with responses at or above median and 22.8 for patients with responses below median (Cox p=0.2580).

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For the KLH control patients, a statistically significant survival advantage was observed for patients with an IgG anti-KLH response below the median compared to those with responses at or higher than the median (30.2 versus 22.3

5   months respectively, Cox p=0.0215).

ITT Overall Baseline CA 27.29 Survival Impact

A baseline serum CA 27.29 level was assessed for patients

10   in both treatment groups (THERATOPE® vaccine and KLH control) utilizing the TRUQUANT BR radioimmunoassay kit (Fujirebio Diagnostics Inc.). CA 27.29 is a tumor-associated antigen correlated with disease stage in breast cancer patients, and has been used as a signal of

15   disease progression or recurrence [2,3,4].

A total of 52% of the THERATOPE® vaccine treated patients and 50% of KLH control patients had elevated levels of CA 27.29 at baseline. Patients in both arms of the trial

20   with normal CA 27.29 experienced a clear survival advantage over their counterparts with elevated levels.

In the THERATOPE® vaccine treated group, sera from 515 patients were assayed. Of these, 249 patients had

25   CA 27.29 levels within the normal range, and 266 had elevated levels. Their median survival was 28.7 and 19.2 months respectively (Cox p = <0.0001).

64

In the KLH control group, sera from 500 patients were assayed, with 250 patients in each of the normal and elevated level groups. Patients with a normal CA 27.29 level survived a median 26.6 months, compared to 18.1

5   months in patients with elevated levels (Cox p = 0.0002).

ITT Overall Week 12 CA 27.29 Relative to Baseline

A total of 270 THERATOPE® vaccine and 231 KLH control

10   patients also had CA 27.29 levels measured at week 12, following receipt of four adjuvanted vaccinations. The data was examined to elicit the number of patients in each treatment group with normal or elevated CA 27.29 at week 12. A further examination of the data was conducted

15   to determine the number of patients who experienced a decrease or increase of their serum CA 27.29 levels at week 12, relative to their baseline antigen level classification. The results were similar for both treatment arms and are shown in Table 6.

20   *Stratification Adjustments and Subsequent Efficacy Outcomes*

In order to more fully understand the data, a patient-by-patient review was conducted following the primary

25   analyses. One of the goals of this review was to identify whether any patients had been inadvertently mis-stratified at the time of study enrollment. Data relevant to the determination of appropriate strata assignment was compiled and reviewed in an independent

and blinded fashion by the team of physicians from the
collaborating sponsor companies, EMD Pharmaceuticals and
Biomira. Each physician provided comments regarding the
strata assignment for each patient based upon pre-
5   determined criteria. In terms of concomitant hormone
therapy use, the criteria required that patients must
receive the therapy for a minimum of three months from
the time of treatment arm randomization, and that the
therapy used must clearly be indicated for cancer
10   treatment, and not for symptom control. Mis-
stratifications were identified in all four pre-planned
subsets. The team of physicians approved the final,
adjusted patient assignments, and the subsequent dataset
was termed "fully adjusted".

15   The numbers of patients assigned to a new stratum were as
follows: 31 patients changed to the hormone group, 10
changed to the non-hormone group, 23 changed to the NPD
group, and 12 changed to the NED group. Efficacy
analyses were re-run on this fully adjusted dataset, and
20   are shown in Table 7.

Following stratification adjustments, each of the
efficacy analyses remained non-statistically significant.
One important difference was observed however, between
the results prior to and following the adjustment: the
25   benefit observed for the THERATOPE® vaccine treated
patients within the hormone subset was now seen in both
the NED and NPD strata.

In an effort to understand these observations more fully,
30   a comparison of prognostic variables between the

treatment groups and within the strata was undertaken.
No statistically significant differences were identified
in any of the categories examined, with the exception of
one variable within the NED/no hormone strata

5   combination. In this group, CA 27.29 was significantly
different, and this difference favored the KLH control
patients (15.6% of KLH control patients had elevated
CA 27.29 versus 51.4% of THERATOPE® vaccine treated
patients; Chi sq = 0.0020). When comparing all of the

10 NED patients, regardless of concomitant hormone
treatment, 43% and 21% of THERATOPE® vaccine and KLH
control patients respectively, had elevated baseline
serum CA 27.29.

15 As the number of patients who were NED at study entry was
relatively small (less than 10% of all patients),
subsequent strata based analyses focused primarily on
examining data by hormone use (with disease status
included as an explanatory variable for statistical

20 adjustment).

A total of 180 THERATOPE® vaccine treated patients and
170 KLH control patients were included in the concomitant
hormone therapy strata following the stratification

25 adjustments. For this subset of patients who received
concomitant hormone therapy, those treated with
THERATOPE® vaccine survived a median 35.0 months, while
KLH control patients survived a median 30.7 months (Cox
p=0.1161).

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Figure 1 shows the Kaplan-Meier survival curve for these groups, utilizing the fully adjusted dataset. Although the results are not statistically significant, they are clinically encouraging.

5

The fully adjusted dataset was also examined to determine if a survival difference was observed between THERATOPE® vaccine and KLH control patients who did not receive concomitant hormone therapy. No significant difference

10   was identified (18.7 and 19.6 months respectively, Cox p = 0.2291).

Survival and TDP by Antibody Response for the Fully Adjusted Hormone Subset

15

Survival by antibody response was also examined for the hormone subset of patients utilizing the fully adjusted dataset. The pattern and degree of benefit was similar to that found in the analysis conducted prior to the

20   stratification adjustment and are shown in Table 8.

TDP by antibody response was also examined for the fully adjusted hormone subset. While no statistically significant differences were observed, a trend to benefit

25   was found for THERATOPE® vaccine treated patients in terms of IgG anti-OSM response at or above the median compared to patients with responses below the median (10.6 versus 6.3 months respectively, Cox p=0.0781).

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Interestingly, while the prognostic variables were well
balanced in the THERATOPE® vaccine patients in terms of
KLH response groups, this was not entirely the case when
examining the balance of these prognostic variables in
5   the STn or OSM response groups.

In the case of STn response, 75% of patients with lower
than the median IgG response had visceral metastasis
compared to 67% with responses at or higher than the
10   median (each of the other prognostic variables were
relatively balanced).

In the case of OSM response, several prognostic
indicators were observed that favored patients with
15   responses at or higher than the median, compared to those
with responses below the median. These include fewer
patients with visceral metastases (65% versus 82%), more
patients with normal CA 27.29 at baseline (56% versus
31%), and more patients with ER+ tumors (84% versus 78%).
20   These factors may have contributed to the ability of
patients to develop higher antibody responses to OSM, as
well as to the extent of survival observed. However,
when these three factors were added as explanatory
variables to the Cox model, the survival difference
25   remained statistically significant (Cox p=0.0397).

KLH control patients were also observed to have an
imbalance in prognostic variables between those with
antibody responses at or above median and those with

responses below the median. Specifically, patients with antibody responses below the median had fewer visceral metastases (71% versus 80%) and more frequent normal CA 27.29 (58% versus 41%). When these factors are added

5   as explanatory variables to the Cox model, the survival difference remains statistically significant (Cox p=0.0357).

Survival Updates 1 and 2

10

Updated survival information was analyzed on two occasions,. Update #1 was six months after the endpoint for the data above, and Update #2 was six months after update #1. As the data matured and were analyzed at these

15   additional time points, the efficacy outcomes between the overall ITT treatment groups remained essentially unchanged. These data are shown in Table 9, along with the original data provided for reference.

The survival of the hormone subset from the ITT

20   population is shown in Table 10, along with the identical analyses for the fully adjusted hormone subset. Included in this table is the original data for reference.

Although the statistical outcome for survival for the ITT hormone subset improves marginally over time, the

25   analyses for the fully adjusted hormone subset of patients reached statistical significance at the most recent update (Cox p = 0.0360). These data are illustrated in Figure 2.

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Survival by Antibody Response over Time for the ITT
Treatment Groups: Updates 1 and 2

Survival was also assessed by antibody response over time

5   for the overall ITT patients. The observed statistical
       trend for THERATOPE® vaccine treated patients with
       anti-OSM responses at or greater than the median, was
       lost over time. No significant difference in survival by
       antibody response to STn or KLH was observed at any time

10   point for THERATOPE® vaccine patients.

       KLH control patients with lower than median antibody
       responses to KLH continued to experience a survival
       benefit over those with a response at or higher than

15   median, although the degree of statistical significance
       lessened over time. The data are shown in Table 11.

       Survival by Antibody Response over Time for the Fully
       Adjusted Hormone Subset: Updates 1 and 2

20

       The analysis of survival by antibody response for the
       fully adjusted hormone subset of women yielded
       interesting observations.

25   THERATOPE® vaccine treated patients with responses at or
       higher than the median to OSM, compared to those with
       less than median response to OSM, were observed to have a
       statistically significant survival benefit at the
       original analysis in June 2003. At each of the follow-up

30   survival analyses, this benefit remained statistically

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significant. These data are shown in Table 12, and the
outcome from the most recent survival update (#2) is
illustrated in Figure 3.

A similar outcome did not occur for KLH control patients.
5   Although KLH control patients with lower than median
antibody responses, compared to those with responses at
or greater than the median, were observed to have a
statistically significant survival benefit at the
original analysis time point, the benefit was lost over
10   time. The data are shown in Table 12.

Discussion

Several observations were identified from the exploratory
analyses: 1) patients who receive anti-cancer hormone
15   therapy concomitantly with THERATOPE® vaccine vaccine
have better TDP and survival outcomes than similar
patients treated with KLH control vaccine, 2) no
difference in TDP or survival was identified between
THERATOPE® vaccine and KLH control patients who did not
20   receive concomitant hormone therapy, 3) THERATOPE®
vaccine treated patients who mount IgG anti-OSM antibody
responses at or greater than the median have improved
survival compared to those with less than median
responses, and 4) KLH control patients with less than
25   median IgG anti-KLH responses had improved survival
compared to those with higher responses.

Not understood is the rationale behind the survival
benefit observed for KLH control patients with less than

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median antibody responses compared to their counterparts with higher responses. Patients in the former group were observed to have several prognostic variables in their favor, which could facilitate a better survival outcome,

5   but it remains unclear why this potentially healthier group would develop lower antibody responses to KLH than those with poorer prognostic characteristics.

The beneficial outcome associated with THERATOPE® vaccine

10   and concomitant anti-cancer hormone therapy is not fully understood. One possible explanation for this outcome is that patients who received concomitant hormone treatment may have had a less aggressive form of breast cancer, as disease progression generally did not occur as quickly

15   for these patients as for those who did not receive hormone therapy. This difference allowed a greater opportunity for concomitant hormone therapy patients to receive more THERATOPE® vaccine vaccinations, and therefore increase the likelihood of immune response

20   development against their disease. While the survival benefit may be due, at least in part, to favorable imbalances in prognostic variables, it is also plausible that those same variables may also have identified patients most likely to respond favorably to

25   immunotherapeutic approaches. Additionally, there may be some type of interaction between hormone therapy and THERATOPE® vaccine treatment.

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Glossary of Terms

Exploratory Analysis: analysis that extends beyond the requirements stated in the trial protocol.

5 *Intent To Treat (ITT):* Includes randomized patients according to the treatment group they were randomly assigned, and the strata to which they were originally designated, prior to corrections to the stratification variables that occurred after data review.

10 *Fully Adjusted:* Corrections or adjustments are made to the stratification variables following data review.

ITT Hormone Subset: The total patient population selected for hormone use prior to corrections to the stratification variables.

15 *Fully Adjusted Hormone Subset:* The total patient population selected for hormone use following the corrections to the stratification variables.

Time To Disease Progression (TDP): Time from the date of randomization to the first reported data of disease
20 progression if a patient has progressed or if a patient has not progressed, the date of death or if a patient is alive, the date of last contact or date lost to follow-up.

25 *Survival Time:* The difference in time from date of randomization to death, if deceased, or to date of last contact or date lost to follow-up, if alive.

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Censored Observation: The last date of contact for patients still alive or lost to follow-up.

5 *Cox Proportional Hazards Model (Cox p):* A regression model for modeling survival times. The model assumes that the underlying hazard rate is a function of the independent variables and consistent over time.

Explanatory Variables: (Covariates) Sometimes called
10 independent variables. Variables used to explain the variation in the survival time, for instance disease status, time between diagnosis and metastasis, baseline CA27.29 level, and so on.

15 *Kaplan-Meier Survival:* Estimate of the survival function for nonparametric samples, taking into account censored observations.

Log-Rank Statistic: Test for equality of the survival
20 curves across the strata.

Kaplan-Meier Survival Median: The time at which half the subjects have reached the event of interest (normally either death or disease progression, depending on what is
25 being analyzed), taking into account censored observations.

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Probability: In the context of this analysis, the likelihood that the results seen could have occurred purely by chance.

5   *Statistically Meaningful:*   When the probability is ≤ 0.05.

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Citation of documents herein is not intended as an
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pertinent prior art, or an admission that the cited
documents is considered material to the patentability of
5   any of the claims of the present application. All
statements as to the date or representation as to the
contents of these documents is based on the information
available to the applicant and does not constitute any
admission as to the correctness of the dates or contents
10   of these documents.

The appended claims are to be treated as a non-
limiting recitation of preferred embodiments.

In addition to those set forth elsewhere, the
following references are hereby incorporated by
15   reference, in their most recent editions as of the time
of filing of this application: Kay, Phage Display of
Peptides and Proteins: A Laboratory Manual; the John
Wiley and Sons Current Protocols series, including
Ausubel, Current Protocols in Molecular Biology; Coligan,
20   Current Protocols in Protein Science; Coligan, Current
Protocols in Immunology; Current Protocols in Human
Genetics; Current Protocols in Cytometry; Current
Protocols in Pharmacology; Current Protocols in
Neuroscience; Current Protocols in Cell Biology; Current
25   Protocols in Toxicology; Current Protocols in Field
Analytical Chemistry; Current Protocols in Nucleic Acid
Chemistry; and Current Protocols in Human Genetics; and
the following Cold Spring Harbor Laboratory publications:
Sambrook, Molecular Cloning: A Laboratory Manual; Harlow,
30   Antibodies: A Laboratory Manual; Manipulating the Mouse
Embryo: A Laboratory Manual; Methods in Yeast Genetics:
A Cold Spring Harbor Laboratory Course Manual;

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Drosophila Protocols; Imaging Neurons: A Laboratory Manual; Early Development of Xenopus laevis: A Laboratory Manual; Using Antibodies: A Laboratory Manual; At the Bench: A Laboratory Navigator; Cells: A Laboratory

5 *Manual; Methods in Yeast Genetics: A Laboratory Course Manual; Discovering Neurons: The Experimental Basis of Neuroscience; Genome Analysis: A Laboratory Manual Series ; Laboratory DNA Science; Strategies for Protein Purification and Characterization: A Laboratory Course*

10 *Manual; Genetic Analysis of Pathogenic Bacteria: A Laboratory Manual; PCR Primer: A Laboratory Manual; Methods in Plant Molecular Biology: A Laboratory Course Manual ; Manipulating the Mouse Embryo: A Laboratory Manual; Molecular Probes of the Nervous System;*

15 *Experiments with Fission Yeast: A Laboratory Course Manual; A Short Course in Bacterial Genetics: A Laboratory Manual and Handbook for Escherichia coli and Related Bacteria; DNA Science: A First Course in Recombinant DNA Technology; Methods in Yeast Genetics: A*

20 *Laboratory Course Manual; Molecular Biology of Plants: A Laboratory Course Manual.*

All references cited herein, including journal articles or abstracts, published, corresponding, prior or otherwise related U.S. or foreign patent applications,

25 *issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein*

30 *are also entirely incorporated by reference.*

Reference to known method steps, conventional methods steps, known methods or conventional methods is

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*not in any way an admission that any aspect, description
or embodiment of the present invention is disclosed,
taught or suggested in the relevant art.*

5   *The foregoing description of the specific
embodiments will so fully reveal the general nature of
the invention that others can, by applying knowledge
within the skill of the art (including the contents of
the references cited herein), readily modify and/or adapt
for various applications such specific embodiments,*

10   *without undue experimentation, without departing from the
general concept of the present invention. Therefore,
such adaptations and modifications are intended to be
within the meaning and range of equivalents of the
disclosed embodiments, based on the teaching and guidance*

15   *presented herein. It is to be understood that the
phraseology or terminology herein is for the purpose of
description and not of limitation, such that the
terminology or phraseology of the present specification
is to be interpreted by the skilled artisan in light of*

20   *the teachings and guidance presented herein, in
combination with the knowledge of one of ordinary skill
in the art.*

        *Any description of a class or range as being useful
or preferred in the practice of the invention shall be*

25   *deemed a description of any subclass (e.g., a disclosed
class with one or more disclosed members omitted) or
subrange contained therein, as well as a separate
description of each individual member or value in said
class or range.*

30   *The description of preferred embodiments
individually shall be deemed a description of any*

80

possible combination of such preferred embodiments,
except for combinations which are impossible (e.g,
mutually exclusive choices for an element of the
invention) or which are expressly excluded by this
5   specification.

If an embodiment of this invention is disclosed in
the prior art, the description of the invention shall be
deemed to include the invention as herein disclosed with
such embodiment excised.

10   . The invention, as contemplated by applicant(s),
includes but is not limited to the subject matter set
forth in the appended claims, and presently unclaimed
combinations thereof. It further includes such subject
matter further limited, if not already such, to that
15   which overcomes one or more of the disclosed deficiencies
in the prior art. To the extent that any claims encroach
on subject matter disclosed or suggested by the prior
art, applicant(s) contemplate the invention(s)
corresponding to such claims with the encroaching subject
20   matter excised.

All references, including patents, patent
applications, books, articles, and online sources, cited
anywhere in this specification are hereby incorporated by
reference, as are any references cited by said
25   references.

Table 1   ITT TDP and Survival by Strata (months)

Strata	Median TDP			Median Survival		
	THERATOPE® vaccine	Control	Cox p-value	THERATOPE® vaccine	Control	Cox p-value
NED / No Hormone	2.9 (n=40)	2.7 (n=38)	0.8882	22.5 (n=40)	27.3 (n=38)	0.1070
NED / Hormone	15.8 (n=16)	16.5 (n=18)	0.9529	41.1 (n=16)	Not reached (n=18)	0.8845
NPD / No Hormone	2.8 (n=315)	2.8 (n=306)	0.4291	18.9 (n=315)	19.0 (n=306)	0.6604
NPD / Hormone	8.1 (n=152)	5.6 (n=143)	0.4882	34.3 (n=152)	30.1 (n=143)	0.2035

Table 2   ITT TDP and Survival by Hormone Group (months)

Strata	Median TDP			Median Survival		
	THERATOPE® vaccine	Control	Cox p-value	THERATOPE® vaccine	Control	Cox p-value
No Hormone	2.8 (n=355)	2.8 (n=344)	0.5233	19.2 (n=355)	20.5 (n=344)	0.4084
Hormone	8.2 (n=168)	5.7 (n=161)	0.4779	34.9 (n=168)	30.7 (n=161)	0.2228

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Table 3   ITT Overall and by Hormone Group Antibody
Responses at Week 12

	Week 12 Median Titers					
	Anti-STn IgG	Anti-STn IgM	Anti-OSM IgG	Anti-OSM IgM	Anti-KLH IgG	Anti-KLH IgM
Theratope	20480	10240	320	1280	20480	80
Theratope H	20480	10240	320	1280	20480	80
Theratope NH	10480				20480	160
Control	0	0	0	0	81920	1280
Control H	0	0	0	0	81920	1280
Control NH	0	0	0	0	81920	1280

Table 4   ITT Overall Median Survival by Week 12 IgG
Antibody Response (months):

ITT	THERATOPE® vaccine N=364			KLH Control N=323
	a-STn IgG	a-OSM IgG	a-KLH-IgG	a-KLH IgG
= median response	31.4 (n=208)	31.2 (n=240)	31.2 (n=205)	23.9 (n=204)
< median response	26.3 (n=156)	24 (n=124)	26.3 (n=159)	33.3 (n=119)
Cox p-value	0.4116	0.0896	0.3183	0.0010

("a-" means "anti-").

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Table 5   Survival by Antibody Response in the Hormone
Subset of Patients (months)

Hormone subset	THERATOPE® vaccine N=133			KLH Control N=117
	a-STn IgG	a-OSM IgG	a-KLH-IgG	a-KLH IgG
≥ median response	41.1 n =79	39.6 n =93	35.4 n =80	29.3 n =70
< median response	32.9 n =54	24.0 n =40	38.2 n =53	NR n =47
Cox p-value	0.1408	0.0033	0.7579	0.0102

Table 6   ITT Overall CA 27.29:   Change from Baseline to
Week 12

Treatment Group	Status at Baseline	Change at Week 12 Relative to Baseline	
		Decrease N (%)	Increase N (%)
THERATOPE® vaccine	Elevated	46 (33.8)	90 (66.2)
	Normal	36 (26.9)	98 (73.1)
KLH Control	Elevated	41 (35.3)	75 (64.7)
	Normal	27 (23.5)	88 (76.5)

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Table 7   Fully Adjusted TDP and Survival by Strata
(months)

Strata	Median TDP (months)			Median Survival (months)		
	Ther	Control	Cox p value	Ther	Control	Cox p value
NED / No Hormone	2.9 (n=36)	3.0 (n=33)	0.4808	24.7 (n=36)	32.1 (n=33)	0.3219
NPD / No Hormone	2.8 (n=307)	2.8 (n=302)	0.7992	18.2 (n=307)	18.6 (n=302)	0.2954
NED / Hormone	19.5 (n=16)	13.7 (n=16)	0.5991	41.1 (n=16)	27.2 (n=16)	0.3011
NPD / Hormone	8.2 (n=164)	5.7 (n=154)	0.3082	34.3 (n=164)	30.7 (n=154)	0.2085

Ther=THERATOPE® vaccine.

Table 8   Survival by Antibody Response in the Fully

Adjusted Hormone Subset (months)

Hormone subset	THERATOPE® vaccine N=142			KLH Control N=123
	a-OSM IgG	a-STn IgG	a-KLH IgG	a-KLH IgG
= median response	39.6 n=97	41.1 n=81	35.0 n=86	29.3 n=75
< median response	25.4 n=45	35.0 n=61	38.2 n=56	NR n=48
Cox p-value	0.0050	0.2183	0.9390	0.0072

Table 9   Median Survival for ITT Patients Over Time

(months)

ITT	Survival		
	THERATOPE® vaccine (n=523)	Control (n=505)	Cox p value
Original	23.1	22.3	0.9158
Update #1	23.1	22.3	0.8786
Update #2	23.1	22.3	0.8732

Table 10   Median Survival for ITT and Fully Adjusted

Hormone Subsets Over Time (months)

ITT Hormone Subset	Survival (months)		
	THERATOPE® vaccine (n=168)	KLH Control (n=161)	Cox p value
Original	34.9	30.7	0.2228
Update #1	35.4	31.6	0.2080
Update #2	35.1	31.4	0.1386
Fully Adjusted Hormone Subset	THERATOPE® vaccine (n=180)	KLH Control (n=170)	Cox p value
Original	35.0	30.7	0.1161
Update #1	38.2	30.7	0.0769
Update #2	36.5	30.7	.0360

Table 11   ITT Survival by Antibody Response Over Time (months)

| Date | THERATOPE® vaccine (N=364) | | | | | | | | | KLH Control (N=323) | | |
| | a-OSM IgG Median response | | | a-STn IgG Median response | | | a-KLH IgG Median response | | | a-KLH IgG Median response | | |
	<	≥	Cox P	<	≥	Cox P	<	≥	Cox P	<	≥	Cox P
Orig	24.0	31.2	0.0896	26.3	31.4	0.4116	26.3	31.2	0.3183	33.3	23.9	0.0010
Upd 1	24.0	31.4	0.2246	26.4	31.4	0.7595	25.9	31.2	0.4312	32.7	23.9	0.0101
Upd 2	24.0	31.2	0.4179	26.4	31.4	0.8269	25.9	31.4	0.5919	32.7	23.9	0.0455

Table 12 Survival by Antibody Response for the Fully Adjusted Hormone Subset Over Time (months)

	THERATOPE® vaccine (N=142)									KLH Control (N=123)		
	a-OSM IgG Median response			a-STn IgG Median response			a-KLH IgG Median response			a-KLH IgG Median response		
Date	<	=	Cox P	<	=	Cox P	<	=	Cox P	<	=	Cox P
	n=45	n=97		n=61	n=81		n=56	n=86		n=48	n=75	
Orig	25.4	39.6	0.0050	35.0	41.1	0.2183	38.2	35.0	0.9390	NR	29.3	0.0072
Upd 1	25.4	41.1	0.0125	35.0	41.1	0.3610	39.6	41.1	0.9118	43.0	31.6	0.0657
Upd 2	25.4	41.3	0.0147	35.1	41.1	0.3745	38.2	40.8	0.9346	39.5	30.8	0.3387

NR=Not reached.